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Diet, Fatty Acids, and Regulation of Genes Important for Heart Disease

John P. Vanden Heuvel, PhD

Address

Department of Veterinary Sciences and Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University, 226 Fenske Laboratory, University Park, PA 16802, USA.
E-mail: jpv2@psu.edu

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Diets rich in omega-3 polyunsaturated fatty acids (PUFAs), such as alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid, are associated with decreased incidence and severity of coronary heart disease. Similarly, conjugated linoleic acids (CLAs), which are found in meat and dairy products, have beneficial effects against atherosclerosis, diabetes, and obesity. The effects of PUFA and CLA are in contrast to polyacids with virtually identical structures, such as linoleic acid and arachidonic acid (n-6 PUFA). This article discusses the possibility that cognate receptors exist for fatty acids on their metabolites that are able to regulate gene expression and consequently affect metabolic or signaling pathways associated with coronary heart disease. Three major classes of receptors, including G-protein-coupled receptors, nuclear receptors, and tyrosine kinases, are discussed. These receptors are involved in the regulation of gene expression and metabolism of fatty acids.

Introduction

Coronary heart disease (CHD) is the leading cause of death in industrialized countries and is of rising concern worldwide. The relationship between CHD and diet has been studied for nearly 100 years, essentially since the first observation of high-fat and high-cholesterol diets producing atherosclerosis in rabbits [1•,2•]. Epidemiologic studies have demonstrated that diets high in saturated fatty acids and/or cholesterol increase serum cholesterol and risk of developing CHD. Correlations between diet and incidence of CHD across geographic boundaries and among emigrants have also been noted. These discoveries have led to the diet-heart hypothesis, which suggests that dietary saturated fat and cholesterol are the major cause of CHD and atherosclerosis in humans [2•]. Although dietary fat has dominated the diet-heart hypothesis, there are many other foodstuffs and nutrients that may be involved in the etiology of this disease.

Fiber, antioxidants, folic acid, calcium, and carbohydrate content of food have an impact on heart disease and atherosclerosis as well [1•].

Not All Fats Created Equal

The type of fat in the diet, in particular the saturation of the fatty acid component, dramatically impacts CHD. For example, all three major classes of fatty acids (saturated, monounsaturated, and polyunsaturated) increase high-density lipoprotein (HDL) cholesterol in humans; however, saturated fatty acids increase and polyunsaturated fatty acids (PUFAs) decrease low-density lipoprotein (LDL) cholesterol. The increased ratio of LDL to HDL in the case of saturated fats is associated with increased risk of developing CHD. Saturated fatty acids are generally considered atherogenic and increase thrombosis [1•]. *Trans* fatty acids, found in vegetable shortenings and deep-fried food, raise LDL to HDL ratios to a much greater degree than saturated fat [1•]. One potential mechanism by which *trans* fats adversely affect insulin resistance, diabetes, and CHD is by inhibiting essential fatty acid metabolism.

Two PUFAs that cannot be made in the body (and both of which are essential fatty acids) are linoleic acid (LA, an n-3 fatty acid) and alpha-linolenic acid (ALA, an n-6 fatty acid). In conditions of LA deficiency, arachidonic acid (AA) may also be considered essential. Once in the body, LA and ALA may be converted to others PUFAs such as AA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Fig. 1). Although many fats have been associated with increasing the risk of CHD (eg, saturated and *trans* fatty acids), EPA and DHA have been associated with a variety of beneficial health effects. For this reason, diets that are high in ALA, EPA, and DHA have been sought, and these diets include fish oils, flaxseed, mustard seeds, soy beans, walnut oil, and green leafy vegetables.

Polyunsaturated fatty acids are important for maintaining membrane integrity and as precursors to bioactive prostaglandins, which regulate inflammation, blood clotting, and lipid metabolism. Thus, it is necessary to have diets sufficient in PUFAs (n-3 and n-6) to maintain a variety of biologic processes. Positive effects of diets high in n-3 fatty acids include reducing abdominal fat, preventing cardiac arrhythmia, lowering serum triacylglycerol levels, decreasing thrombosis, and improving endothelial function. As noted by Hu and Willett [2•], several studies

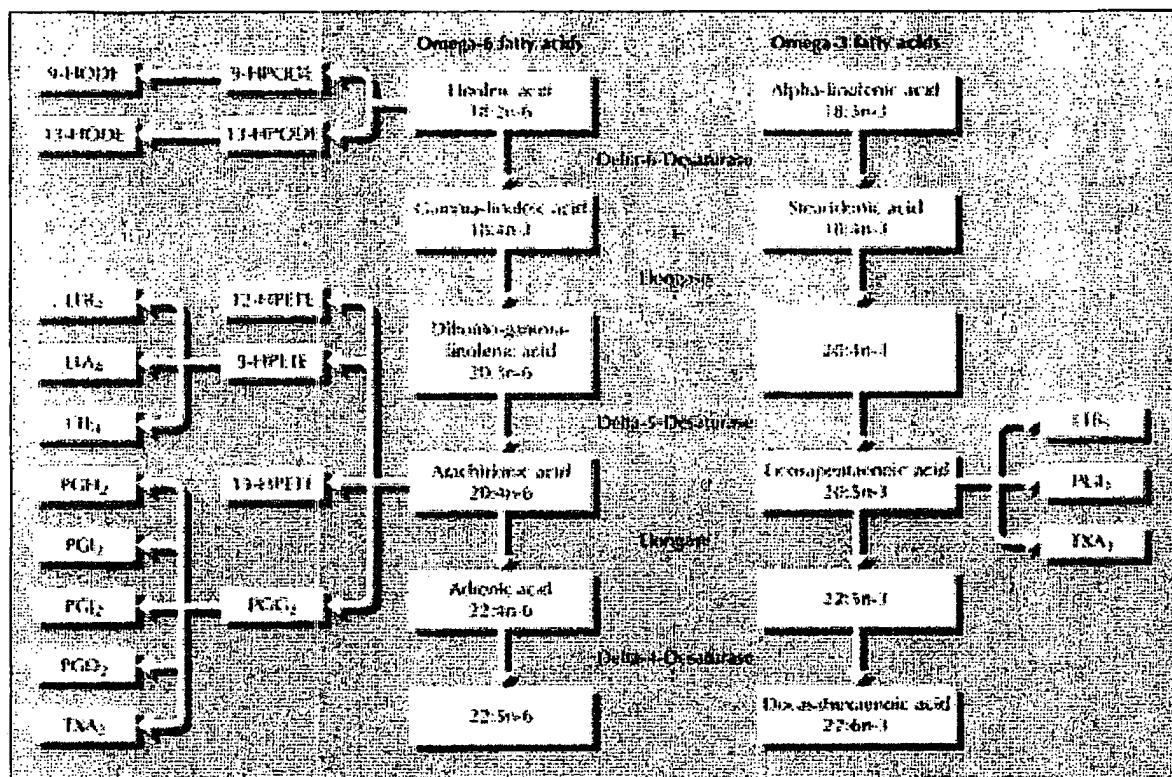


Figure 1. Metabolism of linoleic acid (omega-6 polyunsaturated fatty acid) and alpha-linolenic acid (omega-3 polyunsaturated fatty acid). These fatty acids are thought to be important regulators of coronary heart disease. The fatty acids or their metabolites may be ligands for nuclear receptors such as peroxisome proliferator activated receptor, liver X receptor, and retinoid X receptor, and they may control gene expression. (HODE—hydroxyoctadecadienoic acid; HPETE—hydroperoxyeicosatetraenoic acid; HPODE—hydroxyperoxyoctadecadienoic acid; LT—leukotriene; PG—prostaglandin; TX—thromboxane.)

have shown association of fish intake and/or flaxseed oil (high in ALA) with decreased fatalities from CHD. Importantly, blood levels of EPA and DHA are strongly associated with decreased risk of death, myocardial infarction, and stroke.

Conjugated linoleic acid (CLA) collectively refers to a group of LA derivatives with several positional (double bonds in carbon 9 and 11 or 10 and 12) and geometric (*cis*, *Z* and *trans*, *E*) isomers. CLAs are relatively abundant in ruminant meat and heat-processed dairy products. They are also formed from LA in the intestine of livestock by bacterial flora and are deposited in tissues and milk. CLA has received widespread attention due to its anti-cancer [3-5], antiatherosclerotic [6], and antidiabetic effects [7] in laboratory animals. Whether CLA is metabolized to bioactive molecules such as those noted for ALA and LA has not been determined. However, it is evident from animal studies that CLA has effects on CHD that resemble those of *n*-3 PUFAs.

The question that remains is why some PUFAs, in particular n-3 PUFAs (ALA, EPA, and DHA) and CLAs, are

associated with reduced risk of CHD whereas closely related n-6 PUFAs (LA and AA), and monosaturated (oleic acid) and saturated (palmitic acid) fatty acids are either not as effective or are detrimental to heart health. One explanation may be inhibition of n-6 metabolism by these other structurally similar compounds. This would increase the production of metabolites associated with platelet aggregation, inflammation, and vasoconstriction (leukotriene B₄, prostaglandin [PG] I₂), and thromboxane [TX] A₂) at the expense of those metabolites that have antiaggregation, anti-inflammation, and antivasoconstriction properties (leukotriene B₅, PGI₃, and TXA₃). Another explanation, and the option explored herein, is that cognate receptors exist that preferentially respond to a particular structure of fatty acid. These specific "lipid sensors" would affect gene expression in a tissue-specific, sex-specific, and developmentally specific manner and thereby affect the development of CHD, perhaps by altering enzymes and proteins involved in the transport or metabolism of cholesterol and fatty acids. Also, in order for these receptors to be involved in the beneficial effects of dietary fatty acids, they must be able to

distinguish subtle changes in physical structure of the "good lipids" from "bad lipids," such as n-3 versus n-6 PUFAs, CLA versus LA, and PGI₃ versus PGI₂.

Nuclear Receptors As Sensors of Dietary Lipids

A likely family of proteins that may act as lipid sensors that meet the criteria stated here are the nuclear receptors (NR). Members of the NR superfamily act as intracellular transcription factors that directly regulate gene expression in response to lipophilic molecules [8–13,14•]. They affect a wide variety of functions, including fatty acid metabolism, reproductive development, and detoxification of foreign substances. To date, over 300 NRs have been cloned, many with unknown endogenous ligands (orphan receptors). Phylogenetic analysis has shown six subfamilies (NR1 to NR6) with various groups and individual genes [15]. Several NRs have evolved to respond to dietary lipids (Fig. 2) and include the fatty acid receptors peroxisome proliferator activated receptor (PPAR), retinoid X receptor (RXR), liver X receptor (LXR), and hepatocyte nuclear factor-4 α (HNF4 α) [14•,16]. The receptors shown in Figure 2 may be considered constituents of a large group of NRs known as the "metabolic nuclear receptors," which act as overall sensors of metabolic intermediates, xenobiotics, and compounds in the diet and allow cells to respond to environmental changes by inducing the appropriate metabolic genes and pathways [17••].

Most NRs regulate gene expression in predominantly the same fashion (Fig. 2B). Prior to activation, NRs often exist in multiprotein complexes that vary depending on the family of receptor under question. When a ligand binds to its cognate receptor, a conformational change occurs ("activation") that changes the protein-protein interfaces of the molecule. As a result, the activated receptor interacts with a NR response element (NRE) within the regulatory region of a target gene; upon recruitment of various transcriptional coactivators and subsequently RNA polymerase II (polII), initiation of transcription of the target gene occurs.

In the following sections, the three likely candidates for NRs that respond to dietary fatty acids (*ie*, PPAR, RXR and LXR) are described. The dietary and metabolic intermediates that activate these receptors (Table 1) as well as the genes regulated by these NRs that contribute to prevention of CHD (Fig. 2) are emphasized.

Peroxisome proliferator activated receptors

Of the several identified fatty acid receptors, perhaps the family that can best explain the effects of n-3 PUFAs and the CLAs are the PPARs. The PPAR receptors were originally named based on their ability to respond to xenobiotics (peroxisome proliferators); however, they were also the first to be examined as a fatty acid receptor. It has now been well established that PPAR is a ligand-activated transcription factor involved in gene expression in a tissue-, sex-, and species-dependent manner [14•,17••,18,19•]. The PPARs

exist as three subtypes (α , β , and γ) that vary in expression, ligand recognition, and biologic function.

Peroxisome proliferator activated receptor α was the first transcription factor identified as a prospective fatty acid receptor [20–22]. Based on numerous studies from the PPAR α knockout (PPAR α ^{-/-}), this receptor plays a role in the regulation of an extensive network of genes involved in glucose and lipid metabolism. In particular, PPAR α regulates fatty acid transport; fatty acid binding proteins; fatty acyl-coenzyme A (CoA) synthesis; microsomal, peroxisomal, and mitochondrial fatty acid oxidation; ketogenesis; and fatty acid desaturation.

Several groups have implicated saturated and unsaturated fatty acids as natural ligands for PPAR α [23]. Natural PPAR α ligands in human serum include palmitic acid, oleic acid, LA, and AA. Notably, PPAR α is the only PPAR subtype that binds to a wide range of saturated fatty acids. The 9z 11e CLA isomer is a potent PPAR α ligand with a dissociation constant (K_d) in the low nanomolar range [24], and it affects PPAR-responsive enzymes including acyl-CoA oxidase (ACO), liver fatty acid binding protein (L-FABP), and cytochrome P450 4A1 (CYP4A1) [25]. Similar to other PUFAs, the effects of CLA on body composition are seen in the PPAR α -null mouse [26], suggesting that this NR is not the key target for this response.

Triglyceride-rich lipoproteins, including very low-density lipoproteins (VLDL) and LDL, contain PPAR α ligands [27,28]. Activation of PPAR α is seen when lipoprotein lipase (LPL) is added to VLDL, showing that the endogenous ligands are probably fatty acids or their metabolites esterified into triacylglycerols. Metabolism of AA by CYP4A results in a variety of PPAR α ligands, including 5,6, epoxyeicosatrienoic acids (EET): 8,9 EET; 11,12 EET; 14,14 EET; 20-hydroperoxy-eicosatetraenoic acid (20-HETE); and 20-, 14-, and 15-hydroxyepoxyeicosatrienoic acids (HEET) [29]. Leukotriene B₄ has also been reported to be a selective PPAR α ligand [30]. PGD₂ and PGD₁ activate PPAR α in transient transfection reporter assay systems [31]. The lipoxygenase metabolite 8(S)-HETE is a high-affinity PPAR α ligand, although it is not found at sufficient concentrations in the correct tissues to be characterized as a natural ligand. Because no single high-affinity natural ligand has been identified, Willson *et al.* [23] have proposed that one physiologic role of PPAR α may be to sense the total flux of fatty acids in metabolically active tissues.

Peroxisome proliferator activated receptor γ is expressed in many tissues, including adipose, muscle, vascular cells, macrophages, and epithelial cells of the mammary gland, prostate, and colon [32]. Activated PPAR γ induces LPL and fatty acid transporters (CD36) and enhances adipocyte differentiation, as well as inhibiting cytokine and cyclooxygenase-2 (COX-2) expression, perhaps by modulating nuclear factor- κ B (NF κ B) function. The PPAR γ -null mouse is nonviable, implicating an important role for this protein in ontogeny [33] and also making the examination of a role for this receptor in gene expression difficult.

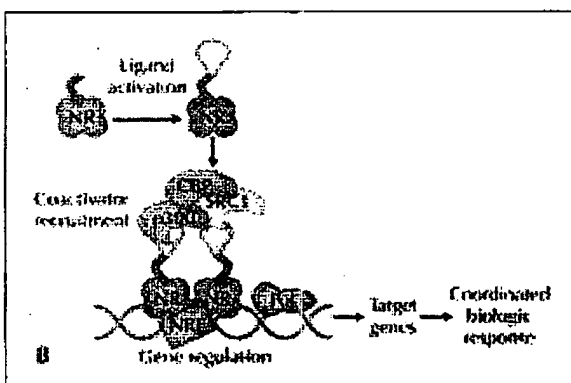
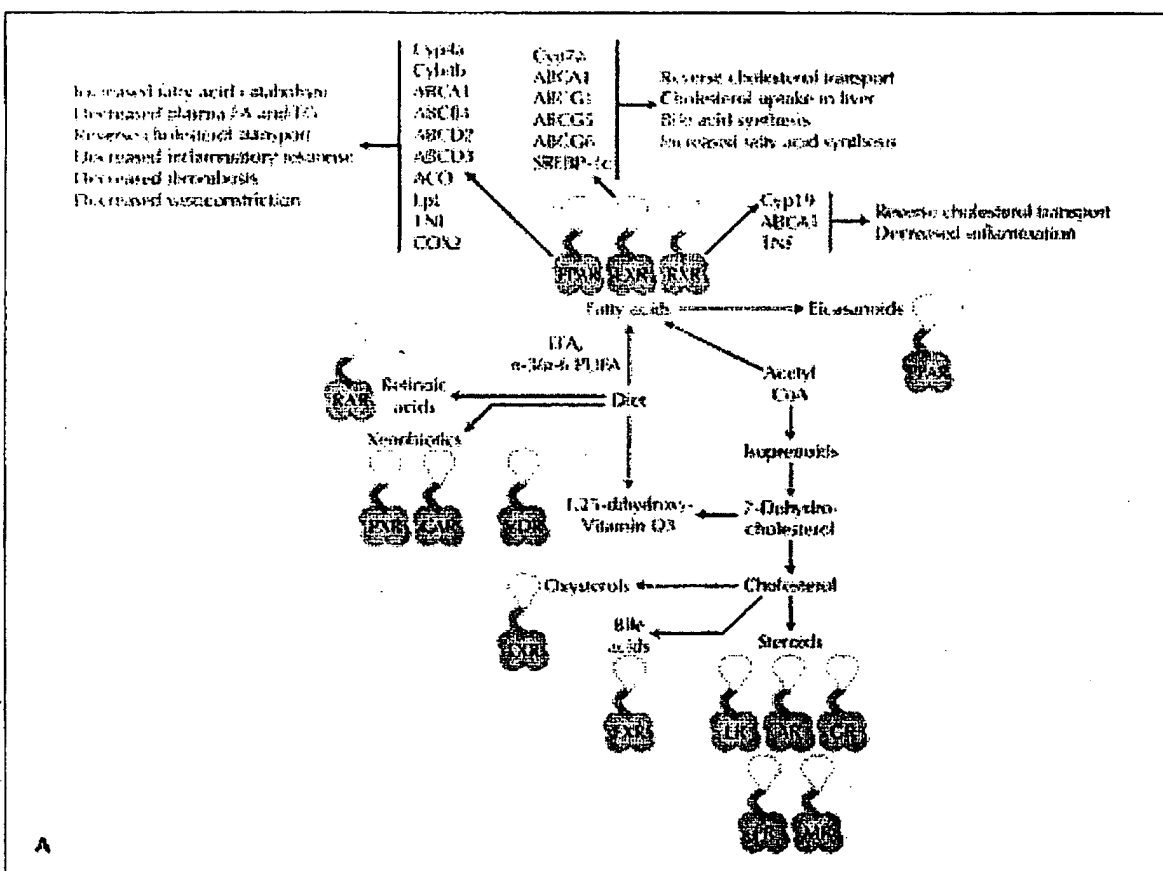


Figure 2. Dietary control of gene expression by nuclear receptors.

A, Nuclear receptors involved in responding to dietary components and intermediary metabolism. The genes and coordinated biologic responses regulated by the fatty acid receptors PPAR, LX, and RXR are shown. B, Mechanism of action of nuclear receptors in regulation of gene transcription. (ABC—ATP binding cassette transporter; ACO—acyl-coenzyme A oxidase; AR—aromatase; CAR—constitutive androstane receptor; COX-2—cyclooxygenase 2; CPB—CREB-binding protein; CYP—cytochrome P450; EFA—essential fatty acid; ER—estrogen receptor; FA—fatty acid; FXR—farnesoid X receptor; GR—glucocorticoid receptor; LPL—lipoprotein lipase; LXR—liver X receptor; MR—mineralocorticoid receptor; NR—nuclear receptor; NRE—NR response element; Pol—RNA polymerase; PPAR—peroxisome proliferator activated receptor; PR—progesterone receptor; PUFA—polyunsaturated fatty acid; PXR—pregnenane X receptor; RAR—retinoic acid receptor; RXR—retinoid X receptor; SRC1—steroid receptor coactivator-1; SREBP—sterol regulatory element binding protein; TG—triacylglycerol; TNF—tumor necrosis factor; VDR—vitamin D receptor.)

Clinically relevant antidiabetic agents such as pioglitazone and rosiglitazone are potent PPAR γ agonists (K_d in low nanomolar range). A number of fatty acids and eicosanoid derivatives bind and activate PPAR α in the micromolar range [30]. Unlike the PPAR α subtype, PPAR γ has a clear preference for PUFAs. The fatty acids LA, AA, and EPA bind PPAR γ within the range of concentrations of

free fatty acids found in human serum [34]. Although fatty acids are not particularly efficacious activators of PPAR γ , intracellular conversion of fatty acids to eicosanoids through enhanced expression of 15-lipoxygenase greatly increased PPAR γ -mediated transactivation [34]. CLA isomers, in particular 9Z11Z and 10E12Z CLA, are ligands for PPAR γ [35]. In macrophages, CLA decreased expression

Table 1. Endogenous and dietary ligands for fatty acid receptors PPAR, LXR, and RXR

Nuclear receptor	Ligand
PPAR α	Saturated and unsaturated fatty acids Omega-3 fatty acids Conjugated linoleic acids LPL-treated VLDL VLDL 5,6, EET; 8,9 EET; 11,12 EET; 14,14 EET; 20,14,15-HEET 2-arachidonylglycerol; 15-S-HETE-G Long chain alkylamines 8-S-HETE PGD ₂ , PGD ₁ Leukotriene B ₄
PPAR γ	Saturated and unsaturated fatty acids Mono- and polyunsaturated fatty acids from triglycerides Conjugated linoleic acids LPL-treated VLDL VLDL PGA ₁ , PGD ₂ , PGD ₁ OxLDL, 9-S-HODE, 13-HODE 15-S-HETE
PPAR β	Polyunsaturated acids including linoleic acid, linolenic acid, arachidonic acid, and eicosapentaenoic acid Conjugated linoleic acid Lysophosphatidic acid Hexadecyl azelaic phosphatidylcholine 13-S-HODE, 15-S-HETE, 5-S-HETE, 12-S-HETE PGD ₁ , PGD ₂ , PGA ₁
LXR	Unsaturated fatty acids (antagonists) Polyunsaturated fatty acids have little effect on LXR activity 22(R) hydroxycholesterol, 20(S)-hydroxycholesterol, 24(S), 25-epoxycholesterol 6 α -Hydroxy bile acids Cholestenic acid Oxysterol 5,6-24(S),25-diepoxycholesterol
RXR	Saturated and mono-unsaturated fatty acids Polyunsaturated fatty acids, including docosahexaenoic acid Conjugated linoleic acids 9-cis retinoic acid Phytol metabolites

EET—epoxyeicosatrienoic acids; HEET—hydroxyepoxyeicosatrienoic acid; HETE—hydroperoxyeicosatetraenoic acid; HETE-G—hydroxyeicosatetraenoic-glycerol ester; HODE—hydroxyoctadecadienoic acid; LPL—lipoprotein lipase; LXR—liver X receptor; OxLDL—oxidized low-density lipoprotein; PG—prostaglandin; PPAR—peroxisome proliferator activated receptor; RXR—retinoid X receptor; VLDL—very low-density lipoprotein.

of proinflammatory signals including COX-2, tumor necrosis factor- α (TNF- α), and inducible nitric oxide synthase (iNOS) in a PPAR γ -dependent manner [36].

Similar to PPAR α , incubation of triglyceride-rich lipoproteins with LPL results in the production of PPAR γ ligands [27,28]. In particular, oxidized LDL (oxLDL) products such as 9-S-hydroxyoctadecadienoic acid (9-S-HODE) and 13-S-HODE are good PPAR γ activators. Phospholipids are also potent PPAR γ ligands, including lysophosphatidic acid (LPA) [37] and hexadecyl azelaic phosphatidylcholine (AzPC) [38].

Peroxisome proliferator activated receptor β (FAAR, NUC1, or PPAR δ) is the least understood of the three subtypes in many respects, including the identification of target genes as well as endogenous and dietary ligands. This receptor is ubiquitously expressed and is often found

in higher abundance than PPAR α or γ . Examination of the PPAR β -null mice has shown a role for PPAR β in development, myelination of the corpus callosum, lipid metabolism, and epidermal cell proliferation [39]. There has been some indication that PPAR β is involved in adipogenesis [39], although this has been refuted [40]. Few high-affinity ligands for PPAR β are known, either xenobiotic or endogenous. However, fatty acids are weak activators of this receptor, with roughly the same preference as PPAR α [23]. CLA isomers, in particular a putative furan metabolite of CLA, activate PPAR β in COS-1 cell transfection experiments [25]. Similar to PPAR α and γ , incubation of triglyceride-rich lipoproteins with LPL results in the production of PPAR β ligands [27,28]. PGA₁, PGD₂, and PGD₁ can activate PPAR β in reporter assays [31].

Role of PPAR in coronary heart disease

The potential of highly potent PPAR activators in the treatment of atherosclerosis has been noted by other investigators [17•, 18, 41, 42•, 43, 44]. Both PPAR α and PPAR γ play key roles in regulating fatty acid metabolism, albeit in seemingly opposite directions [45, 46]. The result of PPAR α activation in rodent hepatocytes and certain other tissues is a dramatic increase in the peroxisomal enzymes with a modest increase in mitochondrial oxidation of fatty acids. In addition, lipid transport proteins such as FABP and acyl-CoA binding protein (ACBP), as well as genes involved in fatty acid and cholesterol export, are under the control of PPAR α . The targeted disruption of PPAR α results in aberrant lipid metabolism, with fat droplets accumulating in liver cells. Not only is peroxisomal metabolism affected, but also the constitutive levels of mitochondrial β -oxidation are less in the PPAR α -null mouse, showing the importance of this protein in overall fatty acid homeostasis.

The array of genes regulated by PPAR γ in adipocytes is indicative of fatty acid accumulation. This regulation of gene expression is concomitant with increased differentiation of immature adipocytes into mature fat-storing cells [47]. These genes include LPL [48], adipocyte fatty acid binding protein (aP2) [49], and CD36 [50]. Adipocyte-secreted cytokines and hormones such as TNF- α and leptin are also PPAR γ target genes [51, 52]. The genes regulated by PPAR γ in macrophages are similar to those in the adipocyte and include LPL and CD36. Treatment of macrophages with PPAR γ synthetic agonists inhibits the production of several cytokines such as interleukin 1- β and TNF- α and may result in an anti-inflammatory response [53]. Another link between PPAR γ and inflammation is the fact that 15-deoxy PGJ2 (a product of the cyclooxygenase pathway) and nonsteroidal anti-inflammatory drugs are potent activators of PPAR γ [54]. It is unclear what role PPAR β may play in regulating genes involved in CHD at this time.

Retinoid X receptors

Retinoid X receptors are involved in the transduction of retinoid signaling pathway, although their role in regulation of gene expression induced by n-3 PUFAs has garnered increasing attention. RXRs (α , β , or γ) can form homodimers or they may serve as a dimerization partner for other NRs, including retinoic acid receptors (RAR), thyroid hormone receptor, vitamin D $_3$ receptor, and PPARs. As a heterodimerization partner, RXR is involved in regulation of multiple cellular pathways. RXR α and β have ubiquitous distribution, whereas RXR γ is expressed in certain organs such as heart, skeletal muscle, and central nervous system structures.

Although intensely studied for synthetic ligands, little is known of the natural activators of this receptor [55•]. RXR is activated *in vitro* by the vitamin A metabolite 9-*cis* retinoic acid (9-*cis* RA), but the levels of this molecule

in vivo are extremely low. Through reporter assays it was observed that DHA is an RXR ligand [55•]. Docosahexaenoic acid, a structurally related compound, activates RXR with a much higher concentration [55•]. DHA's effect was not observed in other nuclear receptors such as RAR, thyroid hormone receptor, and vitamin D receptor, although as stated previously, this fatty acid activates PPAR α . Recently, several fatty acids including unsaturated, mono-unsaturated, and PUFAs such as AA and DHA have been identified as ligands of RXR, thus confirming the activation observed in reporter assays [56]. The 9E11E CLA isomer was by far the most potent of the CLA isomers at activating RXR α and was comparable to the efficacy seen with 9-*cis* RA [14•]. Phytanic acid, a branched chain fatty acid derived from chlorophyll, has also been reported to activate RXR, albeit weakly [57]. Phytanic acid is capable of adipocyte differentiation and induces aP2 mRNA in 3T3-L1 preadipocytes and may act as a natural retinoid in 3T3-L1 cells [57].

Role of RXR in coronary heart disease

Retinoid X receptor α agonists are capable of reducing atherosclerosis in apolipoprotein E knockout mice, an established experimental model of atherosclerosis [58]. Retinoids are capable of increasing the expression of ABCA1, a gene associated with reverse transport of cholesterol. Cholesterol efflux from peritoneal macrophages was significantly increased in an RXR-dependent fashion [58]. RXR-selective agonists counteract diabetes by decreasing hyperglycemia, hypertriglyceridemia, and hyperinsulinemia [58]. Null mutation of RXR α gene resulted in developmental lethality in mice; they died *in utero* and demonstrated severe myocardial and ocular malformations [59]. The malformations resembled severe vitamin A syndrome, suggesting a physiologic role of RXR α in retinoid responses [59].

Liver X receptors

Liver X receptors (LXR α and LXR β) are transcription factors commonly known as cholesterol sensors [17•, 60, 61•]. Although they are important regulators of transport and metabolism of sterols and fatty acids, whether they are direct sensors of n-3 PUFAs has been questioned. Expression of LXR α is restricted, whereas LXR β is ubiquitously present. LXR α is present in certain organs, namely liver, kidney, intestine, adipose tissue, and adrenals. LXR α and β share a high degree of amino acid similarity (80%) and are considered paralogues; as a result there are very few subtype-specific agonists. Oxysterols, including 24(S), 25-epoxycholesterol, 22R-hydroxycholesterol, and 24(S)-hydroxycholesterol, are natural ligands of LXRs. Unsaturated fatty acids as well as AA and other PUFAs competitively block activation of LXR by oxysterols [62]. This offers a potential mechanism for the ability of dietary PUFAs to decrease the synthesis and secretion of fatty acids and triglycerides in liver [62]. This suppressive effect can be eliminated by

deletion and mutation of LXR responsive elements (LXREs) that are located in the promoter region of SREBP-1c. However, others have shown that the unsaturated fatty acid suppression of SREBP-1 and its targeted lipogenic genes is independent of LXR α [63]. Perhaps the effects of fatty acids on LXR-mediated events are being affected by a direct interaction between PPAR α and LXR α [64]. In fact, several xenobiotic PPAR α ligands antagonize LXR's transcriptional activity [65].

Role of LXR in coronary heart disease

There is increasing interest in LXR agonists, whether dietary or pharmaceutical, in the prevention of CHD [60,61,66,67]. The nonsteroidal LXR agonist GW3965 significantly reduced atherosclerosis in murine models of hyperlipidemia [68]. LXR-mediated genes include those associated with cholesterol and bile acid metabolism (eg, ABCA1, ABCG1, APOE, and CYP7A), as well as those with fatty acid synthesis and regulation (SREBP1c, LPL, FAS). Previous studies showed that activation of PPAR γ induced the expression of LXR α and ABCA1 and removed cholesterol from macrophages [69]. Hence, LXR was considered further downstream than PPAR γ in reducing atherosclerosis.

Liver X receptor α knockout mice were unable to respond to dietary cholesterol and failed to induce cholesterol 7-hydroxylase (Cyp7A), the rate limiting enzyme for bile acid synthesis [70]. This resulted in excessive cholesterol accumulation in the liver followed by impairment of functions. LXR α knockout animals also have altered expression of genes associated with lipid metabolism. Interestingly, LXR β knockout mice were unaffected when challenged with dietary cholesterol [71]. Selective bone marrow knockouts of macrophage LXRs increase atherosclerotic lesions in ApoE $^{-/-}$ and LDLR $^{-/-}$ mice, suggesting a role as an endogenous inhibitor of atherosclerosis [68].

Conclusions

Diets high in n-3 fatty acids have long been associated with decreased risk of CHD. ALA and its metabolites EPA and DHA are found in high concentrations in flaxseed and fish oils and are thought to improve heart health through decreasing thrombosis, inflammation, and plaque formation in arteries. The mechanism of these effects may be the result of regulation of gene expression via NRs, several of which are known to be "fatty acid receptors". PPAR α and PPAR β are receptors for unsaturated, mono-unsaturated, and poly-unsaturated fatty acids, as well as for several AA metabolites. Activation of PPAR α is associated with increased fatty acid catabolism, decreasing inflammation, and stimulating the reverse cholesterol pathway. PPAR γ has a clear preference for PUFAs and is also the target of AA metabolites. This receptor is involved in storage of lipids in adipocytes as well as in decreasing inflammation and stimulating the reverse cholesterol pathway. RXR is an important heterodimerization partner for NRs and can affect numerous

metabolic pathways. DHA and several other PUFAs bind to and activate this central NR. LXR's role as a sensor of fatty acids is somewhat controversial, although it is clearly an oxysterol receptor. Several studies have shown that fatty acids (unsaturated and saturated) antagonize LXR activity. This receptor is involved in fatty acid synthesis, bile acid synthesis, and reverse cholesterol transport; synthetic agonists are being touted as antiatherosclerosis agents. Taken together, these NRs represent potential targets for n-3 PUFAs that can help explain their mechanism of action in preventing CHD. In particular, the profile of beneficial effects of ALA, EPA, DHA, and CLA most resemble those seen for synthetic PPAR γ ligands such as rosiglitazone. This connection warrants further critical examination and may ultimately result in modifying diet recommendations to maximize PPAR γ activation, and hence decrease the incidence and severity of CHD.

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Coronary heart disease: dietary links and pathogenesis

Serge Renaud^{1,*} and Dominique Lanzmann-Petithory²

¹INSERM, Unity 330, University Bordeaux 2, 146, rue Léo Saignat, 33076 Bordeaux Cedex, France;

²Department of Internal Medicine, Nutrition and lipid metabolism. Henri Mondor Hospital, 51. Avenue, Marechal de Lattre de Tassigny, 94010 Creteil Cedex, France

Abstract

For decades it has been postulated that the main environmental factor for coronary heart disease (CHD) was the intake of saturated fatty acids (SFA). Nevertheless, confirmation of the role of SFA in CHD through intervention trials has been disappointing. It was only when the diet was enriched in n-3 fatty acids that CHD was significantly prevented, especially cardiac death.

In addition to n-3 fatty acids, many other foodstuffs or nutrients such as fibers, antioxidants, folic acid, calcium and even alcohol contribute to prevent CHD. Thus the relationship between diet and CHD morbidity and mortality appears to be much more complex than formerly suspected considering as key factors only SFA, linoleic acid, cholesterol and atherosclerosis. Some of the mechanisms are briefly described, but many additional nutrients (or non nutrients) may also play an important role in the pathogenesis of CHD.

Finally, as a result of the most recent epidemiologic studies the ideal diet may comprise: 8% energy from SFA, 5% from polyunsaturated fatty acids with a ratio 5/1 of linoleic/alpha-linolenic acid+longer chains n-3, oleic acid as desired, large intake of cereals, vegetables, legumes and fruits, fish twice a week, cheese and yogurt as dairy products, rapeseed and olive oils as edible fat. Without side effects, such a diet can be highly palatable, easily enjoyed by many populations and may prevent effectively and rapidly (within a few weeks or months) CHD.

Keywords
Coronary heart disease
Diet
Saturated fatty acids
Linoleic acid
Alpha-linolenic acid
Trans fatty acids
Antioxidants
Fibers
Calcium
Alcohol

Introduction

Pathologists at the end of last century (Virchow, Ignatowski, Anitschkow) have observed that in human atherosclerotic lesions, there were large amounts of cholesterol deposits. When they fed rabbits with human food including cholesterol, they observed in arteries, lesions somewhat similar to human atherosclerosis. That was the starting point of the diet-heart hypothesis.

Saturated fatty acids, cholesterol and CHD: epidemiology

Cross-population studies

Already in 1930's it was known that all populations with a high intake of saturated fats, presented atherosclerosis and coronary heart disease (CHD). However it was the Seven Countries Study¹ that clearly demonstrated that saturated fatty acids (SFA) were the main environmental factor for CHD.

Moreover, the Japan - Honolulu - San Francisco study², confirmed that the relationship between the intake

of fat and CHD mortality was not due to genetic factors but rather to the diet per se. Many other epidemiologic studies (cited elsewhere³) confirmed the specificity of the association between diet and CHD.

In the Seven Countries Study, the most recent report indicates that even after 25 years follow-up⁴, the CHD mortality is still closely and directly related to the intake of saturated fats. All the individual SFA, lauric (12:0), myristic (14:0), palmitic (16:0) and even stearic acid (18:0) were positively and significantly related to CHD mortality ($r = 0.81$ to 0.86 , $p < 0.001$). Nevertheless, because the intake of each of the SFA was closely related to the total intake of SFA, it was not possible to confirm that all these fatty acids (FA) were independently related to CHD. But the results were clear concerning the total fat intake versus that of SFA. Only the SFA were closely related to CHD. That demonstration is illustrated by the comparison between Crete and East-Finland, the two extreme in hard coronary events, 26 in Crete and 1 074 in East-Finland (age standardized rate per 10 000). The total intake of fat was 40% of energy in Crete and 39% in East-Finland, while the intake of SFA was 8% in Crete and 22% in East-Finland:

*Corresponding author: Email serge.renaud@bordeaux.inserm.fr

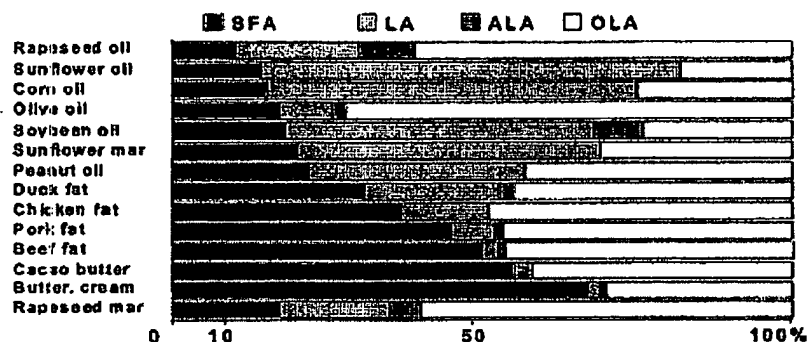


Fig. 1 Fatty acid composition of common fats. Rapeseed mar: rapeseed margarine used in the Lyon study^{9,10}. SFA = Saturated fatty acids LA = Linoleic acid, ALA = Alpha-linolenic acid, OLA = Oleic acid

Within-population studies

They have the disadvantage of a limited variation between individuals in their habitual dietary intake. Their advantage is to be able to adjust for confounding factors such as blood pressure, smoking, body weight and others, that contribute to CHD. As reviewed recently⁵, out of 10 of these studies, 5 have observed a positive significant relationship between the intake of SFA and CHD including the recent Belgium study on 21 500 subjects⁶.

In addition, in the Western Electric Study⁷, even if CHD was not significantly and independently related to the intake of SFA, it was related to the Keys equation that includes SFA, polyunsaturated fatty acids (PUFA) and cholesterol in the diet. In the Zutphen study in Netherlands, no significant relationship was found with the intake of SFA but there was an association with the intake of cholesterol⁸. Thus, it can be concluded that the majority of prospective epidemiologic studies have observed a close relationship between the intake of SFA (or cholesterol) and CHD. For these reasons, the intake of SFA should be reduced to approximately 8% of energy, the level in Crete¹ (the greatest life expectancy in the Western World), in Japan (the greatest life expectancy in the World) and in the recent intervention trial^{9,10} in Lyon.

Dietary fat and CHD: experimental studies

Atherosclerosis

As reviewed elsewhere¹¹, experimental studies have been conducted in animals to determine to what extent dietary fats, especially saturated fats, could induce atherosclerosis and CHD. However in many studies, saturated fats were associated with a high concentration of cholesterol, making it difficult to evaluate the specific contribution of the different fats, especially the respective role of FA. Results were also different depending on whether different natural oils were compared with multiple differences in the level of fatty acids as well as in their position in the glycerol molecule. FA in the sn-2 position

of dietary triglycerides are preferentially absorbed through the intestinal wall while those in sn-1,3 position are released in the intestinal tract and partly excreted in the feces through the formation of calcium soaps¹². Thus, FA in position 2 of dietary triglycerides play a crucial role in the metabolism and biologic effects of these FA as we have shown recently¹³. It may explain observations that did not appear logical without this knowledge. As an example, it may explain why lard seems to be much more atherogenic than reflected by its whole FA composition (Fig. 1). Contrarily to many other fats, more than 65% of the palmitic acid contained in lard is in sn-2 position. This palmitic acid from lard is readily absorbed and metabolized which is not the case for many other animal fats (Fig. 2).

To solve those problems in experimental studies in animals, FA were esterified by methanol instead of glycerol. The results obtained in rabbits indicate that methyl stearate was more atherogenic than methyl oleate¹⁴.

In further studies, Kritchevsky and Tepper, to evaluate the atherogenic effect of individual SFA given under the form of natural dietary fats, interesterified corn oil with either 12:0, 14:0, 16:0 or 18:0. The results were fats with the same amount of SPA in the 3 positions of the triglycerides. Under those conditions in rabbit, all SFA were more atherogenic than corn oil, the most atherogenic being 12:0 and 16:0. Of interest was that 18:0 was associated with a lower serum cholesterol than corn oil, but was more atherogenic.

In addition to rabbits, many other animal species have been used to induce atherosclerosis. It seems that the most relevant to human may be the studies in monkeys. Among the most recent studies are those in African Green monkeys fed 40% of energy as fat, comparing the effect of lard to safflower oil (n-6 FA) or to menhaden oil (n-3 FA)¹⁵. These studies confirmed that lard (saturated fat) was associated with higher serum cholesterol and much higher severity of intimal lesions than safflower and especially menhaden oil.

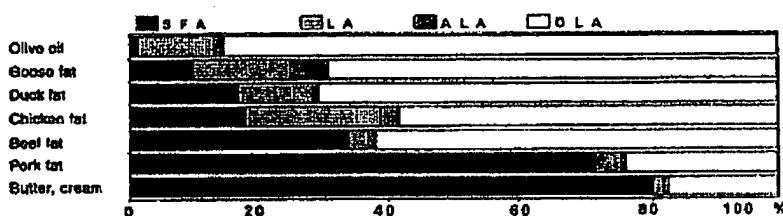


Fig. 2 Fatty acids in sn-2 position of common fats. Abbreviations as in Fig. 1. In sn-2 position pork and dairy fats are very similar, extremely rich in SFA in sn-2 position. By contrast, poultry fats have mostly OLA in sn-2 position. Thus, poultry fats may have comparable atherogenic effects, to that of olive oil, while pork fat and dairy fat may be very similar in terms of atherogenicity

In conclusion, experimental studies in different animal species, as briefly reviewed above, seem concordant in that the most hyperlipidemic dietary fats, in general those rich in SFA, are also the most atherogenic, perhaps with the exception of 18:0. This is an important observation since it has been speculated that the intake of 18:0 may not be predisposing to CHD as the other SFA. The role of 18:0 will be examined further in relation to thrombosis.

Thrombosis

Atherosclerosis induced in animal studies by feeding saturated fats and cholesterol, was in practice never associated with the main human complications of atherosclerosis i.e. coronary thrombosis and sudden death. Specific studies to investigate the relationship between saturated fats and thrombosis have shown consistent results even if only a few investigators have been involved in such studies, years ago.

In 1965, Nordoy, using a standardized damage of the jugular vein, reported that feeding hydrogenated coconut oil and cholesterol to rats increased the incidence of thrombus formation¹⁶. The addition to the diet of linseed oil rich in alpha-linolenic acid (ALA), significantly reduced the incidence of thrombosis, even when thrombosis was initiated by injection of adenosinediphosphate (ADP)¹⁷. In 1969, Renaud and Godu¹⁸, have succeeded in inducing large thrombi by epinephrine or endotoxin, in rats fed a thrombogenic diet rich in SFA. In these studies, it was consistently found that the long-chain SFA (16:0 and 18:0) were the most thrombogenic FA, a result similar to that obtained by Hornstra and Vendelmans¹⁹ also in rat, but in a completely different model. In the Hornstra model the least thrombogenic fat was canola oil, rich in ALA¹⁹.

Contrarily to the atherogenic effects of dietary fats mostly related to their hypercholesterolemic effects, the thrombogenic effect of a fat, at least in the model used²⁰, was neither related to its degree of saturation or its hypercholesterolemic effect. The thrombogenicity of a dietary fat was related to its content in long-chain SFA, specially stearic acid (18:0) the FA the most effective in inducing platelet aggregation and thrombus formation *in vitro* and *in vivo*²¹. The tendency to develop thrombosis in rat was closely associated with platelet hyperaggregability and a shortened clotting time essentially related to

the platelet clotting activity, primarily dependent on the membrane phospholipids. Both the aggregability and the platelet clotting activity were related to the FA composition of the platelet phospholipids²².

In conclusion, in animals it seems that dietary SFA, independently of serum cholesterol, induce a tendency to thrombosis, at least in part through the modification of platelet membranes in their FA composition. In that connection, 18:0 may be the most damaging SFA, a result that has to be confirmed by human studies, directly on CHD, but not only on its effects on blood lipids. In the Lyon study¹⁰, the only SFA significantly lower in plasma of the experimental group markedly protected from CHD, was stearic acid. For these reasons and although further human studies are required in that connection, we cannot consider as confirmed the hypothesis that the intake of stearic acid is safe in relation to CHD, even if it does not increase serum cholesterol.

Sudden cardiac death

Sudden cardiac death is one of the most dramatic clinical manifestations of CHD associated with thrombosis induced ischemia. It is caused by ventricular fibrillation and has been reproduced in dogs²³ and rats^{24,25}. While the risk of fibrillation seems to be increased in rat by feeding meat or animal fat, it can be prevented, both in dog and rat, by infusion or feeding of n-3 FA. Even *in vitro*, on neonatal rat myocytes in cultures, addition of n-3 fatty acids completely inhibited the calcium induced arrhythmia, an effect not reproduced by either SFA or oleic acid (OLA)²⁵. In rat at least, the most efficient n-3 FA to prevent arrhythmia seems to be the precursor of the family, ALA (18:3 n-3), as supplied by rapeseed (canola) oil (n-6/n-3 = 2.6), but not by soybean oil (n-6/n-3 = 9)²⁵. This effect was not reproduced by other oils such as olive and sunflower oils, i.e. by other FA. The mechanism of the antiarrhythmic effects of n-3 FA, in normal and Ca²⁺ overloaded cells, appears to be primarily by reducing the Ca²⁺ entry²⁶.

Trans fatty acids and CHD: Epidemiologic and experimental studies

Trans fatty acids (TFA) can be found naturally in small

amounts (2–7%) in dairy products and meats, but occur mostly in partially hydrogenated vegetable oils.

In the 1940's in USA, two-thirds of the visible fat consumed was from animal and one third from vegetable origin. In the 1960's, two thirds were from vegetable and one third from animal origin.

These changes resulted in a significant increase in the level of TFA both in tub (11–28%) and in hard margarines (19–49%) as well as in shortenings coming from partially hydrogenated vegetable oils²⁷. Those changes occurred under public pressure to reduce the intake of animal and other saturated fats.

Recent evaluation of the TFA consumption in the US put the figure at 8.1 g/person/day²⁷, even 9.6 g representing 2–5% energy. By contrast in Europe²⁸, the intake of TFA ranged from 1.2 g/day in Greece to 6.7 g/day in Iceland. It was 1.6 in Portugal, 2.3 in Finland, 2.4 in Germany, 2.7 in France, 2.8 in UK, 2.9 in Denmark, 4.4 in Belgium and 4.8 in Norway. In Europe, it was only in Finland, Iceland, the Netherlands, Norway and UK that the main source of TFA was partially hydrogenated oils and fats. In the other countries, especially Italy, France and Germany, 75 to 78% of TFA were derived from natural animal sources (milk and ruminant fat).

In recent years, a large prospective study on the effect of TFA in 85 095 female nurses was reported²⁹, with 431 cases of CHD. After adjusting for 11 confounding variables, the relative risk (RR) was 1.16 (95% CI 0.81–1.55), for the second quintile compared to the first quintile; 0.99 (0.69–1.43), for the third; 1.16 (0.80–1.70) for the fourth and 1.47 (0.98–2.20) for the fifth quintile, with a significant trend ($p = 0.0006$) for increasing risk. Therefore in this study, there was a significant positive association between the intake of TFA and CHD.

It was further estimated³⁰ that an increase of 2% of energy from TFA would result in a 93% increase in the risk of CHD while a 5% increase in SFA would only result in a 17% increase risk of CHD. Such an association has not been found in other studies³¹ and debates are still going on, on whether TFA are more harmful than SFA.

As to the mechanism involved in the possible deleterious effects of TFA on CHD, all the recent clinical studies indicate that TFA consumption (from 3.8 to 11.2% energy) is associated with increased concentration of serum cholesterol and LDL cholesterol, with an unchanged HDL cholesterol or slight decrease. At the same time, a decrease in the ratios of LDL/HDL or serum cholesterol/HDL cholesterol was noted. In addition, four recent studies reported that TFA were increasing Lp(a) concentration³¹.

As to whether TFA influence the severity of atherosclerosis in animal studies, seven different studies were reported by Nicolosi³² in rabbits, pigs and vervet monkeys. In none of these studies there was any increase in the risk of atherosclerosis whatever was the level of TFA included in the diet (from 3.2% or 6.0% trans in the

diet with 14% fat given to vervet monkeys³³ to 50% TFA in the 17% fat diet given to pigs³⁴). Thus in experimental models of atherosclerosis tested, there was little evidence that dietary TFA caused the development of atherosclerosis, even fatty streaks. Therefore, there is a striking difference, from the experimental point of view, between the atherogenic effect of SFA and that of TFA. Of course, negative effect does not mean that there is no effect. For example in the studies with monkeys³³ there was very little difference in serum cholesterol between the controls and the animals given the TFA diet. Thus in that study a higher level of atherosclerosis could not be expected in the group with TFA. On the other hand in the rabbit studies of Weigensberg *et al.*³⁵ the animals given TFA had a higher level of cholesterol in blood but the severity of atherosclerosis was similar to that of controls.

As concluded by Nicolosi³² additional studies in various animal models for long periods of time are needed because of the chronic nature of atherosclerosis. In addition, further experimental work should include effects on platelets, thrombosis and ventricular fibrillation.

In conclusion, as emphasized by Martijn Katan³⁶ the fact that a few epidemiologic studies found a positive association between TFA and CHD deserves attention. 'By themselves these data fall far short of proving that high intakes of TFA promote CHD but the effects of TFA on plasma lipoproteins lend some credence to a causal link'. Nevertheless, since the intake of TFA in Europe is much lower than in USA, especially than in the Nurses Health Study^{29,30} (0.5–2.1% energy vs 2 to 5%), the intake of TFA in Europe may not be as harmful as it has been found in USA. In addition, it is also a possibility that the TFA, derived from natural animal sources as it is the case in many European countries, may not be as noxious as the TFA resulting from oil hydrogenation, an hypothesis which has to be confirmed by further studies. In the Lyon diet heart study, TFA supplied less than 1% energy, and spectacular protective effects on mortality and recurrences were observed^{9,10}. Thus we are suggesting that TFA could supply up to 1% of energy, without obvious damaging effects.

Nutrients with protective effects on CHD

Polyunsaturated fatty acids (PUFA)

The linoleic (18:2 n-6) (LA) and alpha-linolenic (18:3 n-3) (ALA) acids are essential for normal growth and physiologic functions of all tissues. These FA have double bonds located respectively at six (n-6) and three (n-3) carbon atoms from the methyl group. Men and animals cannot include a double bond in position n-6 or n-3 i.e. synthesize the corresponding FA. Thus, those two FA are essential. However men and animals can add double bonds to these FA between the original double bonds and the carboxyl group. The carbon chain can also be elongated at the carboxyl end. These metabolic processes

are responsible for the production of long chain (20–22 carbon atoms) metabolites with 3 to 6 double bonds. The result is the formation of two FA families (n-6 and n-3), both essentials, competing for the same enzymatic systems. Thus, as already suggested³⁷, they have to be supplied in food at a proper level, probably as a ratio of 3–5/1, but not 10–20/1 as encountered at present in many countries. Those FA are important components of membrane phospholipids involved in fluidity and deformability of membranes and even in the clotting activity of these membranes³⁸. Among these FA, arachidonic and other FA with 18 to 20 carbon atoms are precursors of potent mediators such as prostaglandins and leukotrienes.

In addition, located in the membranes at the interface between the extracellular and intracellular compartment, they may influence intracellular signaling pathways. Arachidonic acid and its metabolites as a second messenger system, activate transcription factors and induce the expression of early genes as an immediate cellular response, that can be antagonized by the n-3 FA family³⁹. Thus again, a physiologic balance of the two essential FA families has to be supplied in the food in relation to health in general. Because in vegetable oils, only LA lowers serum cholesterol as shown by many studies, recommendations to prevent CHD was to increase the intake of PUFA, essentially LA, to 10% of energy, by using oils and margarine containing mostly LA (sunflower oil, safflower oil, corn oil). We have observed⁴⁰ that at a high intake, LA blocks the conversion of ALA in its metabolites, a result confirmed by Emken⁴¹ using deuterium labeled LA and ALA.

Addition of a large amount of LA to a diet low in SFA to decrease serum cholesterol by 15% as in the Minnesota Coronary Survey⁴² did not prevent, in primary prevention, cardiovascular and all cause mortality. Even in secondary prevention, the use of soybean oil containing approximately 60% LA and 8% ALA, was not associated with a protection from cardiac death both in the Leren⁴³ and Morris and Ball⁴⁴ trials.

In rat, soybean oil did not prevent ventricular fibrillation (sudden death)²⁵. Only rapeseed (canola) oil was efficient for that purpose, possibly owing to its ratio LA/ALA of 2.6/1 compared to 7.5/1 in soybean oil. By using rapeseed oil and margarine, we were able to completely prevent sudden death in our secondary prevention trial⁹ the ratio LA/ALA in the whole diet being 5/1. Longer chain n-3 FA from fish oil may also prevent cardiac death to some extent as shown by the diet and reinfarction trial (DART)⁴⁵.

In addition, recent prospective studies have shown that the only PUFA inversely related to CHD was ALA either on non-fatal^{46,47} myocardial infarction (MI) or on cardiac death⁴⁸. In that connection it has been observed that the FA lowering platelet aggregation in man was ALA⁴⁹ but not LA that rather increases platelet response to ADP in rat, monkey and man⁵⁰.

Finally, in the Lyon diet heart study⁵¹, in the experimental group having a much higher intake of ALA, the occurrence of cancers was reduced by 61%, after a follow-up of 4 years. This suggests that in addition to CHD, other serious health problems such as cancer and inflammation⁵², may require a change in the balance of LA/ALA.

Concerning the long chain n-3 FA (EPA, DHA) it has been suggested that the low rates of CHD in Greenland and in Japan may be due to the high consumption of fish in these countries⁵³. This hypothesis has been supported by prospective studies showing in most of them that the subjects with a moderate intake of fish at baseline, had a lower risk of fatal CHD compared to men who did not consume any fish^{54,55}. These observations have been confirmed by intervention trials such as the DART⁴⁵ and the GISSI⁵⁶ trials on the prevention of cardiac death. Mechanisms involved, as reviewed by Leaf and Weber⁵⁷, include a lowering of very low-density lipoproteins (VLDL), of thromboxane production and of blood viscosity associated with an increase in fibrinolytic activity and in prostacyclin synthesis. As already emphasized, the protection from sudden cardiac death may be by reducing the Ca²⁺ entry in myocytes²⁶.

In conclusion, both the n-6 and n-3 FA are essential for life, but have opposite effects on several systems and compete for the same enzymes. Thus for the prevention of CHD and other serious health problems, the dietary supply of PUFA should not be higher than 5% energy with a proper ratio of the two families, probably in the range of 3–5/1 (LA/ALA+longer chain n-3) but not 10 to 20/1 as frequently observed in Western countries.

Monounsaturated fatty acids (MUFA)

The relative failure of LA to prevent CHD in intervention trials⁴² as well as the possible carcinogenic⁵⁸ and suppressive effects on the immune system⁵⁹ have drawn attention to MUFA in the prevention of CHD. The Cretan population with the greatest life expectancy in the Western World¹ and the lowest risk of CHD, has an extremely high intake of MUFA, essentially OLA (9-cis 18:1), as supplied by olive oil.

Earlier studies have considered OLA as a neutral FA⁶⁰ neither raising nor lowering serum cholesterol. This neutral effect also extends to all lipoprotein fractions: VLDL, low density lipoprotein (LDL) and high density lipoprotein (HDL). That neutrality should not be considered as a disadvantage since very few nutrients can be considered totally safe. The concept of the neutrality of OLA can be extended to imply its safety. In terms of carcinogenicity or its effect on the immune system, OLA emerges as one of the safest nutrients⁶¹. Recent analyses⁶² suggest or confirm previous work in that OLA is not totally neutral on LDL-cholesterol, but rather exerts a significant lowering effect which is half as potent as LA. Moreover OLA increases the concentration of HDL-cholesterol⁶³ slightly more than LA⁴⁷. One reason for

OLA to be associated with a lower LDL-cholesterol is that OLA is the preferred substrate for the ACAT (acyl CoA cholesterol acyltransferase). When the liver is enriched with OLA, an increase in the hepatic LDL receptor activity is observed, resulting in a reduction in the LDL-cholesterol production rate and a drop in the LDL-cholesterol level in blood.

In addition to have its concentration reduced by OLA, LDL enriched in OLA by supplement feeding in humans, presented lower proinflammatory properties⁶⁴. That study demonstrates that when exposed to oxidative stress, the LDL enriched in OLA promotes less monocyte chemotaxis (52% lower) and reduces monocyte adhesion by 77% compared with LA enriched LDL. LDL enriched in OLA may be less readily converted to proinflammatory minimally oxidized LDL, able to enhance early events of atherosclerosis such as monocyte chemotaxis and adhesion. Finally, OLA may enhance cholesterol efflux from human fibroblasts⁶⁵.

In addition to protective effects on atherosclerosis per se, an OLA rich diet seems also to have a beneficial effect on thrombogenesis. Compared to SFA rich diet, human subjects on a OLA enriched diet for 8 weeks, exhibited a significantly lower activation of postprandial factor VII and concentration of factor VII antigen⁶⁶. The interest in factor VII is that it is positively associated with CHD mortality⁶⁷ and may be involved in the initiating mechanisms of coronary thrombosis.

Thus considering all the positive effects for health of a diet rich in OLA as briefly described above, it seems logical that such a diet can be associated with a low mortality rate from CHD. Nevertheless, for the type of huge protection observed in Crete (95 to 98% compared to other countries) additional nutrients such as ALA, fibers, antioxidants and others may be required. Nevertheless, because of the safety of OLA as a nutrient, its intake may not be restricted in a healthy diet, except perhaps as a risk for overweight.

Dietary fiber

As reviewed by William Connor⁶⁸, the fiber hypothesis has been developed by two physicians Dr Denis Burkitt and Dr HC Trowell both working at Kampala, in Uganda. They noted that the Africans they treated had rarely Western diseases such as CHD, hypertension, diabetes, cancer and others. They speculated that the reason was that the African diet contained a lot of roughage that had been eliminated of the Western diet.

It was discovered that soluble fibers such as pectin from fruits or beta-glucan in oat bran, lower serum cholesterol⁶⁹. However the effect on cholesterol seems to be extremely modest. Thus, it has been concluded that the main effect of oat bran consumption is to replace the high fat, high cholesterol foods usually consumed.

Nevertheless, recent prospective studies indicate that a higher intake of fibers from fruits, vegetables and cereals

reduces the risk of mortality not only of CHD⁷⁰, but also of cancer and all causes⁷¹.

In Finland⁷² a prospective study on 21 930 smoking men, followed for 6.1 years, has observed that both non-fatal myocardial infarction and coronary death were inversely related to the intake of fiber, the association being higher with coronary death. For men in the highest quintile of dietary fiber (34.8 g/day) coronary death was reduced by 31% ($p < 0.001$ for trend) compared to the lowest quintile (16.1 g/day). Adjustment for confounding variables did not change the results.

The conclusion was that greater intakes of food rich in fiber, independently of other risk factors, substantially reduce the risk of CHD mortality. Soluble fibers and cereal fibers seemed to have the greatest effects.

In USA⁷³, on 43 757 US male health professionals followed for 6 years, fatal coronary disease and non-fatal MI were reduced by 41% among men in the highest quintile of fiber intake (28.9 g/day) compared with men with the lowest quintile (12.4 g/day). As in Finland, adjusting the results for confounding variables did not change much the significance of the data. Also as in Finland, the inverse association was strongest for fatal CHD and the cereal fibers were the most strongly associated with the reduced risk of CHD. As to the mechanism involved, the reduction in CHD observed in these last 2 prospective studies is larger than would be expected from the limited beneficial effect on serum cholesterol, especially for cardiac death. A high fiber intake has been associated with a decrease in the level of insulin and an increase in insulin sensitivity⁷⁴. Effects on hemostatic variables have also been reported⁷⁵.

Further studies are certainly required to elucidate the mechanisms involved in the remarkable protection observed in the prospective studies. Intervention trials seem to be required since the only intervention with fiber (in secondary prevention) did not observe any beneficial effect on CHD⁴⁵. Nevertheless, the intake of fibers is certainly not associated with adverse effects and thus, their consumption should not be restricted but rather largely encouraged.

Antioxidants

The renewed interest for the putative protective effect of antioxidants on CHD⁷⁶ comes from the Steinberg *et al.* hypothesis suggesting that it is the oxidatively modified LDL that are atherogenic. Studies in rabbits show that atherosclerosis can be reduced by adding an antioxidant to the diet, an effect independent of plasma cholesterol⁷⁷. Free radicals are highly reactive molecules because they contain an unbound electron. In our body, they can oxidize many molecules such as lipids, especially unsaturated fatty acids (UFA). Under normal physiologic conditions, cells are protected against free radicals by enzymes (superoxide dismutase, catalase, glutathione peroxidase) and by antioxidants such as vitamin E (the

main antioxidant in membranes), vitamin C and beta-carotene. When the balance between the formation of free radicals and antioxidant defenses is disturbed, UFA from LDL are oxidized. Oxidized LDL become atherogenic eliciting a chemotactic response stimulating monocytes, to be transformed into macrophages and subsequently into foam cells. Oxidized LDL are cytotoxic and damage endothelial cells, stimulating platelet aggregation and procoagulant activity⁷⁸. Finally, the susceptibility of LDL to oxidation appears to be related to the severity of coronary atherosclerosis in man⁷⁹.

In LDL, all UFA are not susceptible to peroxidation at the same extent. LA appears to be much more susceptible than OLA⁸⁰. As to n-3 FA, it has been postulated that the long chain n-3 from fish oil (EPA, DHA) may inhibit rather than increase oxidative modification of LDL⁸¹, despite the number of double bonds in the molecules.

The next step in the antioxidant hypothesis of atherosclerosis is to determine whether dietary antioxidant substances are inversely related to CHD.

Prospective studies in 39 910 male health professionals⁸² and 87 245 female nurses⁸³ have observed that the incidence of CHD was about 40% lower in subjects who consumed vitamin E supplements. By contrast 3 European prospective studies^{84,85,86} in which vitamin E was supplied mostly by food, did not show an inverse relationship to CHD. It has been postulated that the prevention of CHD requires large amounts of vitamins difficult to get from dietary sources alone.

Nevertheless, intervention studies with vitamin E supplements have not clarified further the problem. In the 'Cambridge Heart Antioxidant Study' (CHAOS)⁸⁷ study on secondary prevention of CHD, 2 002 patients were randomly assigned to a pharmacological dose of vitamin E or placebo. After 17 months, vitamin E reduced non fatal MI by 66% and cardiac death+MI by 36% respectively, in such a way that there was a non-significant increase in cardiovascular death in the treated group. In the ATBC trial in Finland, 50 mg daily of vitamin E did not reduce CHD mortality or the incidence of angina pectoris in heavy smokers⁸⁸.

Thus there are considerable doubts as to whether we know the dose and duration of vitamin E supplementation to effectively prevent CHD and whether a diet rich in vitamin E might not be more efficient than supplements. In that connection, it has been shown that a high concentration of vitamin E can act as prooxidant⁸⁹, especially in the absence of aqueous antioxidants such as ascorbate and urate⁹⁰. Polyphenols can be added as powerful antioxidants that also can regenerate vitamin E.

An example of the possible role of other antioxidants on the vitamin E status was the French adaptation of the Cretan diet⁹. Despite a lower intake of vitamin E, the experimental group had a significantly higher plasma level of vitamin E at one year, related to the intake and the plasma level of vitamin C. Vitamin C is known to protect

and regenerate vitamin E⁹¹ and the polyphenols may have a similar effect. Nevertheless, the intake of vitamin C was not inversely related with CHD, in the large prospective studies from Harvard^{110,111}, contrarily to polyphenols in Netherlands⁹² and Finland⁹³ found to be beneficial.

To be noted is that the Cretan cohort (the greatest life expectancy in the Western World) had with the Corfu cohort the highest intake of vitamins C and E of the Seven Countries Study⁹⁴, out of their local food. Although there is absolutely no doubt that in the human diet a high intake of vegetables and fruits with their antioxidant components, is associated with a consistent protection from CHD and other diseases, further studies are warranted to evaluate under what conditions antioxidant supplements may have similar beneficial effects. By contrast, in all populations with a high intake of vegetables and fruits, as well as in all studies having investigated the effects of such diets, beneficial effects were observed not only on CHD but also on other diseases. Thus, a high consumption of vegetables and fruits should be encouraged by any means.

Folic acid, pyridoxine (vitamin B6)

It has been known for years⁹⁵ that an increased level of homocysteine in blood was associated with severe arteriosclerosis. Homocysteine is an amino acid produced in the metabolism of methionine, usually not detectable in plasma or urine. As a result of a genetic disorder, large amounts accumulate in plasma and urine and these patients die early of CHD. Even a moderately increased level of homocysteine in blood is associated with increased risk of peripheral vascular disease, CHD and stroke⁹⁶.

Enhanced platelet aggregation, endothelial damage⁹⁷ and lipid peroxidation⁹⁸ have been postulated as possible mechanisms in the predisposing effect of homocysteine to CHD.

To avoid accumulation of homocysteine in blood, homocysteine has to be converted to cysteine in a vitamin B6 dependent reaction or remethylated to methionine in a relation involving folic acid and vitamin B12. One efficient way to reduce homocysteinuria appears to be through administration of folic acid alone, or folic acid and pyridoxine combined⁹⁹. In further studies, only folic acid or the combination with the B vitamins led to a significant reduction of homocysteine in plasma, but not vitamins B12 or B6 per se¹⁰⁰.

In a recent prospective study, Rimm *et al.*¹⁰¹, have observed, in 80 082 nurses after 14 years of follow-up, that the number of non fatal MI and fatal CHD were reduced by 45% in the highest quintile of both folic acid and vitamin B6 intake compared with the lowest quintile. 26% of folate was supplied by multiple vitamins and the rest by cereals, orange juice, lettuce, eggs, broccoli and other vegetables and fruits. A surprising result in this study was that the protective effect of folate and vitamin

B6 was much more efficient in women consuming more than one drink of alcohol per day.

It has also been reported that n-3 fatty acids from fish oil reduced the level of homocysteine compared to olive oil¹⁰². Thus it may be that other nutrients than vitamins may have an effect on homocysteine, a topic that requires much further studies.

Calcium

The interest of dietary calcium in relation to CHD is that in many countries, an inverse relationship has been found between hard water and the occurrence of cardiovascular diseases^{103,104}. This effect appears to be due to the calcium content of the waters¹⁰⁵.

In man dietary calcium is known to induce a borderline reduction of serum cholesterol¹⁰⁶ but an 18% decrease in triglycerides has been noted¹⁰⁷. In studies on 9 groups of French and British farmers the intake of calcium evaluated by the chemical analysis of a duplicate sample of food including water consumed, was inversely related in multivariate analysis, to the level of triglycerides but not of cholesterol¹⁰⁸. In the studies in man¹⁰⁸ as well as in rabbits¹⁰⁹, the intake of calcium was strongly inversely related to thrombin induced platelet aggregation and platelet clotting activity. It seems that the intake of calcium was regulating the absorption of saturated fats and their effect on platelets. Possible mechanisms¹¹⁰ could be that dietary calcium promotes the excretion of SFA in the feces, especially the long chain SFA (16:0, 18:0) the most active to promote platelet aggregation and susceptibility to thrombosis in animals.

The National Health and Nutrition Examination Survey in USA, showed that a low calcium intake gives rise to high blood pressure¹¹¹. In the Nurses Health Study¹¹², results obtained were similar. An intake of 800 mg calcium decreased the risk of high blood pressure compared to an intake of less than 400 mg/day.

The National Institute of Health Continuing Medical Education recommends a daily intake of up to 2 000 mg of calcium per day to prevent hypertension. At that level, additional beneficial effects on triglycerides and platelet aggregation may be expected in subjects with a high intake of SFA. Thus, in all studies evaluating the relationship between the intake of SFA and occurrence of CHD, the intake of calcium from food and water has to be considered as a confounding factor in addition to the other risk or preventive factors.

Cheese are the richest foodstuffs in calcium but is also rich in SFA. Nevertheless, Greece and France, the highest consumers of cheese in the world, enjoy the highest life expectancy in the Western World. Studies suggest that the absorption of SFA from fermented dairy products (cheese, yogurt...), through the formation of insoluble calcium salts of SFA, partly excreted in the feces, is much lower than from whole milk, a structure designed for total absorption of all nutrients. In the Lyon study^{9,10} and also

in the Finnish experiment¹¹³, consumption of cheese and yogurt was suggested, if possible, low in fat. Thus, consumption of cheese seems to be compatible with a low mortality rate from CHD, probably through the beneficial effect of calcium.

Finally, in all populations, an increased intake of calcium from food and water seems to be a justified recommendation for health.

Alcohol

The inverse relationship between morbidity and mortality from CHD and the moderate consumption of alcoholic beverages, has been documented by ecological, case-control and prospective studies involving more than 1 million subjects¹¹⁴. Depending on the prospective studies, with an intake of 2 to 4 drinks per day, reduction in CHD mortality was of 20 to 60%. Only one study in Finland has shown an increase risk, whatever was the intake of alcohol¹¹⁵. It has to be emphasized that in this Finnish study, 60% of the alcohol was spirits, that it was used for intoxication (binge drinking) and was followed by sudden death or stroke. Binge drinking of beer seems to have similar effect¹¹⁶ since after heavy acute intake a 6.5 fold increase in fatal MI was observed. It is known that alcohol could be beneficial for CHD only if used moderately and spread out over the week.

Rimm *et al.*¹¹⁷ found that the average number of days per week on which alcohol was consumed was inversely associated with the risk of CHD. Men who reported drinking on 3-4 days per week had a 34% reduced risk of CHD compared with men who drank on less than one day. Thus, to have protective effect on health, particularly to prevent CHD, alcohol has to be used as in the Mediterranean countries, mostly at meals, through the week, and of course, moderately.

A question frequently raised is whether wine per se is healthier than other alcoholic beverages. On CHD as emphasized by Rimm *et al.*¹¹⁷, data so far do not confirm that wine protects more efficiently than other alcohols. In our recent prospective study in Eastern France¹¹⁸ on 36 250 men, we found that the protective effect of wine may be more consistent than that of beer. It is mostly on cancer and death from all causes, at least in man, at very moderate intake, that wine appears to offer more protection than the other alcohols as also observed by Gronbaek *et al.*^{119,120}, probably owing to phenolic compounds such as resveratrol¹²¹, contained in wine.

Concerning the mechanisms involved in the protective effect of alcohol, it has been associated with an increased level of HDL-cholesterol, independently of physical activity¹²². However, it seems that the effect on HDL can explain only 50% of the protective effect of alcohol¹²³.

To protect from CHD, the main target of alcohol seems to be MI i.e. coronary thrombosis rather than atherosclerosis per se^{124,125}. In that connection the influence of alcohol drinking on platelet aggregation was examined in

Diet and coronary heart disease

467

rat as well as in man and shown to be markedly reduced after moderate intake. However, under acute ingestion of ethanol, a rebound effect on platelet response can be observed that may explain the untoward effects of binge drinking resulting in sudden death and stroke¹²⁶. This rebound effect on platelets does not seem to occur with wine drinking at least in rats, owing to the antioxidant polyphenols¹²⁷. It is known that alcohol drinking increases peroxidation that appears to be related to the rebound effect. The polyphenols from wine impede that peroxidation and the untoward effects of ethanol on platelets¹²⁵.

Concerning the long-term effects of alcohol on platelets in man, we found in 1 600 subjects from Wales, that the intake of alcohol in a dose-related manner, was inversely associated with the response of platelets, most significantly secondary aggregation to ADP¹²⁸, probably through thromboxane A2 production¹²⁹. This is exactly what aspirin does¹³⁰, known also for its remarkable protective effects on CHD¹³¹. Thus, it is no longer surprising that a moderate intake of alcohol could be associated with a protective effects on CHD since it increases the level of HDL-cholesterol at about the same extent as physical activity¹²², and reduces platelet aggregation similarly to aspirin. Nevertheless, depending on the type of alcoholic beverage wine, beer, spirits and the way it is used (binge drinking or moderate consumption regularly at meals) opposite effects can be observed on CHD and other health problems. Thus recommendations should be similar to the 1995 US dietary guidelines for Americans: 'If you drink alcoholic beverages, do so in moderation, with meals and when consumption does not put you or others at risk'¹³².

Dietary fatty acids and serum cholesterol

As shown in human and several animal species, feeding increasing amounts of cholesterol alone results only in modest increases in serum LDL-cholesterol or total cholesterol. By contrast, with a constant intake of cholesterol, increasing the amount of a triacylglycerol containing predominantly SFA, results in an increased response of serum cholesterol. This effect is suppressed if UFA replace the SFA of the triacylglycerol.

Thus, these observations indicate that cholesterol and LDL-cholesterol in blood are increased to some extent by the intake of cholesterol, but that it is predominantly the amount and type of fat which determine their blood level. To determine whether all SFA were equivalent to increase cholesterol in blood, FA from 6:0 to 18:0 were fed to hamsters¹³³. The shorter chain FA (6:0 to 10:0) did not significantly elevate LDL-cholesterol in blood, compared to dietary cholesterol alone. These FA are rapidly metabolized to acetyl Co A in the liver, and do not change the FA composition of the lipid pools. By contrast the 12:0, 14:0 and 16:0 FA markedly increased the

LDL-cholesterol production and its concentration in plasma. By contrast, 18:0, although it became enriched in liver, did not change the production rate of 18:0 and did not alter the plasma level of LDL-cholesterol. Similar results were obtained in human studies. Thus it seems that the only FA biologically active concerning LDL-cholesterol are 12:0, 14:0 and 16:0, while 18:0 is biologically neutral.

When 18:1 (9-cis) (OLA) was given to animals in the same studies as above, the LDL-cholesterol production rate was reduced as well as the LDL-cholesterol concentration. By contrast, the same FA with a trans configuration 18:1 (9-trans) (Elaidic acid) did not lower the plasma level of LDL-cholesterol.

Because early studies suggested that LA was more potent than OLA to lower serum cholesterol when compared to SFA, the first equations to calculate the effects of dietary FA on plasma total cholesterol, considered only SFA and PUFA.

Keys *et al.*¹³⁴: $\Delta TC = 1.2 (2 \text{ SFA} - \text{PUFA})$, 18:0 being excluded from SFA.

Hegsted *et al.*¹³⁵: $\Delta TC = 2.10 \text{ SFA} - 1.16 \text{ PUFA}$

Recently these equations have been revisited taking into account the lowering effect of OLA (MUFA) and even of (18:0) stearic acid

Mensik and Katan¹³⁶: $\Delta TC = 1.51 \text{ SFA} - 0.12 \text{ MUFA} - 0.60 \text{ PUFA}$

$\Delta LDL - c = 1.28 \text{ SFA} - 0.24 \text{ MUFA} - 0.55 \text{ PUFA}$

Derr *et al.*¹³⁷: $\Delta TC = 2.3 (14:0) + 3.0 (16:0) - 0.8 (18:0) - 1.0 \text{ PUFA}$

$\Delta LDL - c = 2.6 (14:0) + 2.9 (16:0) - 0.5 (18:0) - 0.7 \text{ PUFA}$

All these equations have been obtained in metabolic ward studies and are all consistent with our present knowledge. Nevertheless, they only give indications on the short term level of serum cholesterol (and LDL-cholesterol) that can be expected from the dietary habits. They are not able to give the real risk of CHD since there are many additional factors involved in that risk.

TC=Total cholesterol

LDL-c=LDL-cholesterol

Dietary prevention of CHD

Once dietary risk factors are discovered, mechanism of their effects elucidated, before public health recommendations it still remains to test, in randomized intervention trials, the dietary factors involved to be reasonably certain of their eventual beneficial effects. It is only intervention trials that offer the best chance of directly answering whether or not diet can reduce the risk of CHD and other diseases. Soon after the discovery of the positive relationship between saturated fats and CHD, intervention trials were set up to verify through a relatively simple diet manipulation whether saturated fats were really the villain. In the 1960's two comparable secondary prevention

trials were organized to prevent recurrences and death in 400 coronary patients each and lasted 5 years. The Leren trial⁴³ observed a significant lowering of coronary events but not of total mortality or sudden death. The Morris trial⁴⁴ although similar to the Leren trial in terms of diet modification and lowering of cholesterol (16% for Morris vs 14% for Leren) did not observe a significant reduction either of coronary events or of cardiac or total mortality. The diet recommendations were to decrease as much as possible the intake of saturated fats and to use mostly soybean oil for preparing food. Apparently, the subjects were sticking to the diet since their cholesterol was lowered similarly in the 2 trials. In these studies, the polyunsaturated/saturated ratio (P/S) was increased from 0.2–0.3 to more than 1.8 i.e. a large intake of PUFA to replace SFA.

A conclusion of these studies was that in secondary prevention, once the patients had already CHD, it was too late to try to prevent by dietary manipulations coronary events and death since atherosclerosis is a long lasting pathologic process. Thus, additional trials in primary prevention were set up as briefly shown in Fig. 3. Of special interest was the primary prevention of the Minnesota Coronary Survey because the dietary changes were similar to the previous secondary trials with a P/S ratio of 1.6 in the experimental group compared to 0.3 in the control group⁴². Also, it was a double blind

randomized trial on 9 057 subjects in psychiatric hospitals in Minnesota i.e. the ideal conditions for a trial. After 4.5 years of follow-up, serum cholesterol was lowered by 15% in the experimental group compared to controls, similarly to the Leren and Morris secondary trials.

Concerning coronary events and all-cause mortality, they were increased by 8% in the experimental group. These results do not suggest that diet, especially dietary SFA, are not related with CHD but rather that the type of diet proposed, not used by any population in the world, was not better to prevent CHD than the diet used in Western Countries at that time.

In the meanwhile, Hjermann and Leren in Oslo had settled an additional trial, this time in primary prevention, but in subjects at risk because they smoked and were hypercholesterolemic¹³⁸.

The diet intervention was combined with a decrease in smoking but apparently the effect was due mainly to the dietary habits. In the experimental group coronary events were reduced by 44%, cardiac death by 59%, sudden death by 69%, all cause mortality by 39% after a follow-up of 102 months. The diet recommendations were to decrease the intake of SFA without being replaced by PUFA, to use more bread, more vegetables, lean meat, more fishes and fruit for dessert. In the first report, the P/S ratio mentioned for the experimental group was 1.01 with 8.2% of calories supplied by saturated fat. After reevaluation,

Prevention through increase in n-6 fatty acids

Name	year	(Ref)	Type, N° subjects	Intervention	Serum chnl	Total mortality	Non fatal MI	P
Leren	1970	(43)	Secondary, 400	↑ P/S (0.3-2.4)	↓ 14%	N.S.	↓ 23%	0.05
Morris	1968	(44)	Secondary, 400	↑ P/S (0.2-1.8)	↓ 16%	N.S.	↓ 16%	N.S.
Woodhill	1978	(148)	Secondary, 400	↑ P/S (0.8-1.7)	↓ 4%	↑ 50%	Not reported	
Hjermann	1986	(138)	Primary, 1232	↑ P/S (0.3-0.7) ↑ n-3 (Fish)	↓ 10%	↓ 39%	↓ 44%	<0.05
Frantz	1989	(42)	Primary, 9057	↑ P/S (0.3-1.6)	↓ 15%	↑ 8%	↑ 8%	N.S.

Prevention through increase in n-3 fatty acids

Hjermann	1986	(138)	Primary, 1232	↑ P/S (0.3-0.7) ↑ n-3 (Fish)	↓ 10%	↓ 39%	↓ 44%	<0.05
Dart	1989	(45)	Secondary, 2033	↑ P/S (0.6-0.6) ↑ n-3 (Fish)	N.S.	↓ 30%		N.S.
Lyon	1994-5	(9,10)	Secondary, 600	↑ P/S (0.7-0.7) ↑ n-3 (ALA)	N.S.	↓ 70%	↓ 73%	<0.001
Gissi	1999	(56)	Secondary, 11324	↑ P/S similar ↑ n-3 (EPA,DHA)	N.S.	↓ 20%		N.S.

Fig. 3 The main dietary intervention trials to prevent CHD. Total and cardiovascular (not shown) mortality were significantly lowered only when the intake of n-3 FA was increased. Non fatal MI may be lowered, primarily when the n-3 FA is ALA (the Lyon study) as also observed in recent prospective studies^{46,47}. Nevertheless in the Lyon and Hjermann trials the higher intake of vegetables and fruits may have contributed to the observed protective effects. P/S = polyunsaturated/saturated fatty acids, chol = cholesterol

Diet and coronary heart disease

469

the P/S ratio was only 0.7³¹ and serum cholesterol lowered by only 10%. Thus the Hjermann trial was the first to really demonstrate a significant reduction in CHD by a somewhat palatable diet more comparable to diet used by populations with low mortality rate from CHD.

Since in the 1980's it was clear that coronary thrombosis was the main factor responsible for myocardial infarction, before undertaking our own trial⁹ we determined first in human populations, what diet was the most efficient to lower platelet reactivity (aggregation, coagulation). As shown in French farmers^{40,108} it was not a diet low in SFA and rich in PUFA, especially in LA even if it was the easiest way to lower serum cholesterol. It was a Mediterranean type diet used in South of France and we reproduced its effects in Moselle, in Eastern France⁴⁰.

The fat composition of the diet to decrease platelet aggregation to all agonists consisted of moderate changes with 10% of energy from saturated fat, a P/S ratio of 0.6 (PUFA, 6% energy) and a ratio of LA/ALA 5/1. It was not far from the diet used in Crete, associated with the lowest mortality rate from CHD among the 16 groups of the Seven Countries¹ and the greatest life expectancy in the Western World¹³⁹. It included a somewhat high intake of ALA as shown by the fatty acid composition of cholesterol esters in Crete compared to Netherlands¹⁴⁰.

In the Lyon trial on 600 coronary patients^{9,10}, to stick more closely to the Crete diet, it was advised to the patients to adhere to the following suggestions¹⁰.

1. more bread,
2. more vegetables and legumes,
3. more fish,
4. less meat (beef, lamb, pork) replaced by poultry,
5. no day without fruit,
6. no more butter and cream, to be replaced by a special margarine made out of rapeseed oil and to switch from sunflower oil or similar oils rich in LA to olive oil or rapeseed oil.

The special margarine used in the intervention trial had been designed already for the Moselle study⁴⁰. Its FA composition (Fig. 1) was very close to that of rapeseed (canola) oil. It is also somewhat similar to that of olive oil.

In Crete, olive oil is used with all meals including breakfast, on bread, with coffee. At present in France, it is difficult to have olive oil with all meals. Thus to have patients accepting to switch from butter and cream to another type of fat, margarine had to be supplied with a composition similar to olive oil. Rapeseed oil was selected because it is the only oil very similar to olive oil but in addition it contains more ALA than olive oil (6–8% instead of 1%) that in Crete is supplied by different foodstuffs such as purslane, snails, walnuts and others.

Concerning dairy products, only cheese, yogurt, fermented dairy products in general, especially low fat, were encouraged. A moderate intake of wine was also suggested.

After a mean follow-up of 27 months, non fatal MI and cardiac death were reduced by more than 70% with the Mediterranean type diet compared to controls with a prudent diet. Serum cholesterol, triglycerides, HDL and LDL cholesterol, Apoprotein A and B, Lipoprotein(a) were identical in the 2 groups.

In addition, there were 8 sudden deaths in the control group and none in the experimental. Additional thrombotic events such as unstable angina, stroke and thromboembolism were also reduced by more than 70%. By contrast stable angina, more closely related to the severity of atherosclerosis, thus to serum cholesterol, was only reduced by 28% in the recent evaluation after 4 years¹⁴¹.

It can be considered if confirmed, that the Lyon diet heart study reproduces, in France, the striking protection of the Cretans for CHD and also all cause mortality, essentially with an adaptation of their diet since medical treatment was identical in the two groups. As in Crete compared to other Mediterranean groups¹, serum cholesterol in Lyon was not lower in the experimental group than in the control^{9,10}. In the experimental group, the intake of saturated fat was lower (8% of energy vs 10.6%) especially stearic acid, as confirmed by the FA composition of plasma. The intake of OLA was higher by 40% and the ratio of LA/ALA was 23/1 in controls versus 4.5–5.0/1 in the experimental group with an ALA intake of 2 g/day. The experimental group also consumed more bread, legumes, vegetables and fruits, while fish consumption was similar in the two groups.

A key factor in the protection observed in the experimental group may be the intake of ALA¹⁴¹, probably similar to that in Crete as evaluated from the level in plasma¹⁴⁰. Also of interest is that a high level of ALA has also been found in Japan¹⁴² and in Canadian Inuits¹⁴³, two populations known for their protection from CHD usually attributed to fish consumption. It has also been found in 3 prospective studies as the only fatty acid inversely related to non fatal^{46,47} or fatal MI⁴⁸. ALA supplied under the form of rapeseed oil, was the most efficient FA at least in rat, to prevent ventricular fibrillation and sudden death^{24,25}. We found that it was also the only FA associated with lower platelet reactivity⁴⁰ in confirmation of the pioneer work of Owren on platelet adhesiveness¹⁴⁴.

The work of Singh *et al.*, in India, obtained results comparable to the Lyon diet heart study to prevent recurrences after an MI, with a diet rich in vegetables and fruits¹⁴⁵ or with n-3 FA¹⁴⁶. Finally, in Finland, between 1972 and 1992, the sharp decline in CHD appears to be the result of changes in the main risk factors, attributed to diet modification.

In brief, these dietary changes consisted:

1. in switching from high fat to low-fat milk, now used by 80% of the population.

2. replacing butter on bread by soft margarines,
3. using rapeseed (canola) oil for cooking and in the margarine industry,
4. increasing continuously over the years the consumption of fish and cheese,
5. increasing consumption of fruits (x2) and vegetables (x3).

Thus the changes in the dietary habits that took place in Finland during the last part of this century were very similar to those of the experimental group in the Lyon diet Heart Study.

Concerning the public health implications of the diet recently designed to effectively prevent CHD, it is interesting to note that such dietary changes at the level of the Finnish population have been associated with an enjoyable decline of 55 to 68% in the mortality rate from CHD over a period of 20 years. During that period, CHD mortality in Finland, once the highest in the Western World, declined markedly (up to 80% in 40–50 years old men) in such a way it is now similar to that of UK or USA¹⁴⁷. Apparently it is more than can be explained by the main risk factors¹¹⁵ i.e. smoking, blood pressure and cholesterol, the last two being markedly influenced by diet. Of additional interest is that, at least in intervention trials^{9,145}, these dietary protective effects occur rapidly i.e. within a few months after diet modification, owing to direct effects on thrombosis and cardiac (sudden) death, independently of serum cholesterol as shown by human and animal studies.

In addition to ALA and other n-3 FA, many nutrients may be involved in the protective effects of diet as summarized in Fig. 4. Of additional interest is that such dietary changes were also associated with a drastic

reduction in cancer mortality, both in Finland and in the Lyon trial⁵¹.

Abbreviations

ACAT	acetyl CoA cholesterol acyltransferase
ADP	adenosine diphosphate
ALA	alpha-linolenic acid
CHD	coronary heart disease
DHA	docosahexaenoic acid
FA	fatty acids
EPA	eicosapentaenoic acid
HDL	high density lipoproteins
LA	linoleic acid
LDL	low density lipoproteins
LDL-C	LDL cholesterol
LP(a)	lipoprotein a
MI	myocardial infarction
MUFA	monounsaturated fatty acids
n-3 FA	family of fatty acids derived from alpha-linolenic acid (18:3 n-3)
n-6 FA	family of fatty acids derived from linoleic acid (18:2 n-6)
OLA	oleic acid
PUFA	polyunsaturated fatty acids
P/S	polyunsaturated/saturated fatty acids
SFA	saturated fatty acids
TC	total cholesterol
TFA	trans fatty acids
UFA	unsaturated fatty acids
VLDL	very low density lipoproteins

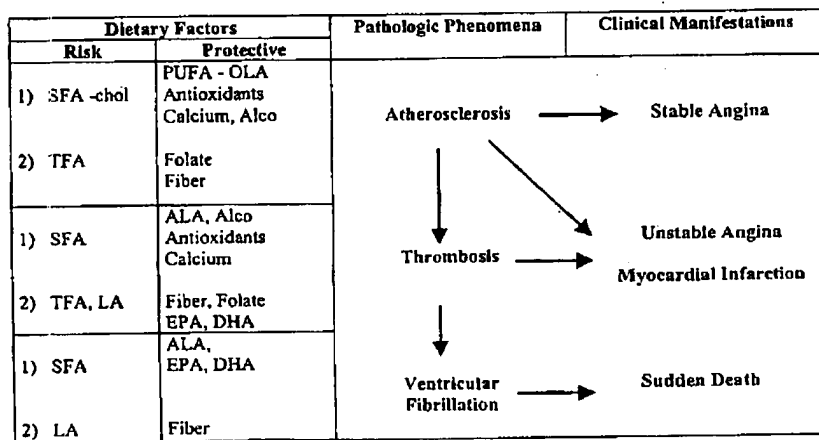


Fig. 4 Nutrients with demonstrated or possible links with CHD clinical manifestations. Evidence: (1) reasonable; (2) to be confirmed. Abbreviations: ALA = alpha-linolenic acid, Alco = alcohol, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, LA = linoleic acid, OLA = oleic acid, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids, TFA = trans fatty acids

Diet and coronary heart disease

471

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Diet and coronary heart disease

473

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CLINICAL CARDIOLOGY

Optimal Diets for Prevention of Coronary Heart Disease

Frank B. Hu, MD, PhD

Walter C. Willett, MD, DrPH

THE RELATIONSHIP BETWEEN DIET and coronary heart disease (CHD) has been studied intensively for nearly a century. In 1908, Ignatowski produced atherosclerosis in rabbits with a diet high in cholesterol and saturated fat¹; feeding the rabbits cholesterol alone produced identical lesions. In the early 1950s, controlled feeding studies demonstrated that saturated fatty acids and, to a lesser extent, cholesterol increased serum cholesterol concentration in humans.² Meanwhile, epidemiologic studies found that increased serum cholesterol predicted risk of CHD in human populations. These discoveries led to the classic diet-heart hypothesis, which postulated a primary role of dietary saturated fat and cholesterol in the cause of atherosclerosis and CHD in humans.³ The diet-heart hypothesis gained further support from ecological correlations relating saturated fat intake to rates of CHD in cohorts from different countries⁴ and from studies of migrants from low- to high-risk countries.⁵

Until recently, most epidemiologic and clinical investigations of diet and CHD have been dominated by the diet-heart hypothesis. However, the original hypothesis was overly simplistic because the effects of diet on CHD can be mediated through multiple biological pathways other than serum total cholesterol or low-density lipoprotein cholesterol (LDL-C) (FIGURE 1).⁶ The existence of these multiple pathways heightens the need to study clinical outcomes because the use of a single in-

Context Coronary heart disease (CHD) remains the leading cause of mortality in industrialized countries and is rapidly becoming a primary cause of death worldwide. Thus, identification of the dietary changes that most effectively prevent CHD is critical.

Objective To review metabolic, epidemiologic, and clinical trial evidence regarding diet and CHD prevention.

Data Sources and Study Selection We searched MEDLINE through May 2002 for epidemiologic and clinical investigations of major dietary factors (fat, cholesterol, omega-3 fatty acids, *trans*-fatty acids, carbohydrates, glycemic index, fiber, folate, specific foods, and dietary patterns) and CHD. We selected 147 original investigations and reviews of metabolic studies, epidemiologic studies, and dietary intervention trials of diet and CHD.

Data Extraction Data were examined for relevance and quality and extracted by 1 of the authors.

Data Synthesis Compelling evidence from metabolic studies, prospective cohort studies, and clinical trials in the past several decades indicates that at least 3 dietary strategies are effective in preventing CHD: substitute nonhydrogenated unsaturated fats for saturated and *trans*-fats; increase consumption of omega-3 fatty acids from fish, fish oil supplements, or plant sources; and consume a diet high in fruits, vegetables, nuts, and whole grains and low in refined grain products. However, simply lowering the percentage of energy from total fat in the diet is unlikely to improve lipid profile or reduce CHD incidence. Many issues remain unsettled, including the optimal amounts of monounsaturated and polyunsaturated fats, the optimal balance between omega-3 and omega-6 polyunsaturated fats, the amount and sources of protein, and the effects of individual phytochemicals, antioxidant vitamins, and minerals.

Conclusions Substantial evidence indicates that diets using nonhydrogenated unsaturated fats as the predominant form of dietary fat, whole grains as the main form of carbohydrates, an abundance of fruits and vegetables, and adequate omega-3 fatty acids can offer significant protection against CHD. Such diets, together with regular physical activity, avoidance of smoking, and maintenance of a healthy body weight, may prevent the majority of cardiovascular disease in Western populations.

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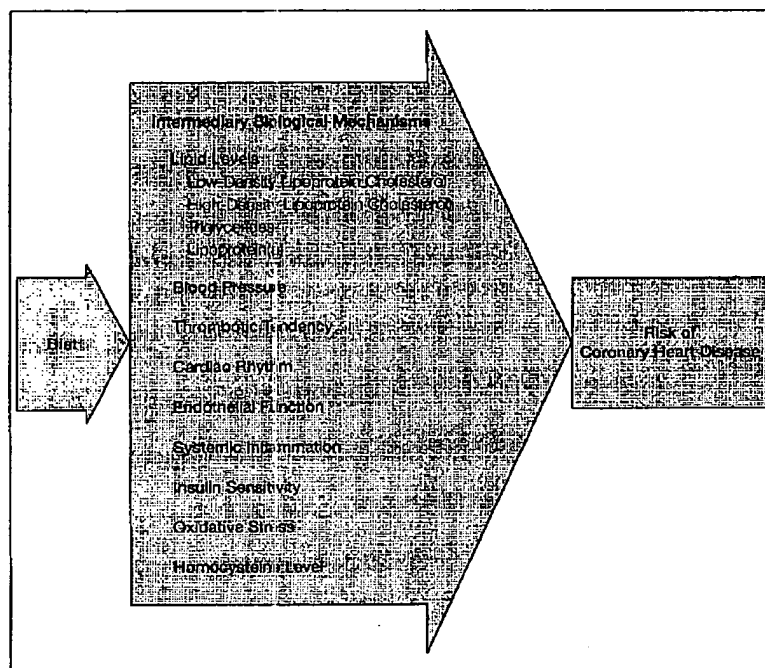
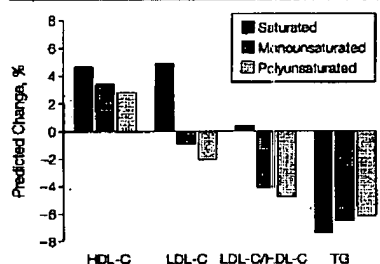
termediate end point as a surrogate of CHD risk could be misleading. In the past 2 decades, understanding of the nutrients and foods likely to promote cardiac health has grown substantially owing to studies of the molecular mechanisms of atherosclerosis and the metabolic effects of various nutrients and foods, large and carefully conducted prospective cohort investigations, and dietary intervention trials. Although the search for the optimal diet

for prevention of CHD is far from over, more specific and firmer evidence on diet and CHD is now available.

Author Affiliations: Departments of Nutrition and Epidemiology, Harvard School of Public Health, and Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Mass.

Corresponding Author and Reprints: Frank B. Hu, MD, PhD, Department of Nutrition, Harvard School of Public Health, 665 Huntington Ave, Boston, MA 02115 (e-mail: frank.hu@channing.harvard.edu).
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OPTIMAL DIETS FOR PREVENTION OF CHD

Figure 1. Mechanisms by Which Diet Potentially Influences Risk of Coronary Heart Disease**Figure 2.** Predicted Changes in Serum Lipids and Lipoproteins

Predicted changes are based on replacement of 5% of energy as carbohydrates with specific fatty acids under isocaloric conditions, assuming baseline high-density lipoprotein cholesterol (HDL-C) levels of 50 mg/dL (1.30 mmol/L), low-density lipoprotein cholesterol (LDL-C) levels of 130 mg/dL (3.37 mmol/L), and triglyceride (TG) levels of 150 mg/dL (1.70 mmol/L).¹⁶

METHODS

For this review, we searched MEDLINE through May 2002 for epidemiologic and clinical investigations of various di-

etary factors (fat, cholesterol, omega-3 fatty acids, *trans*-fatty acids, carbohydrates, glycemic index, fiber, folate, specific foods, and dietary patterns) and CHD. We selected 147 original investigations and reviews of metabolic studies, epidemiologic studies, and dietary intervention trials relating to diet and CHD. Data were examined for relevance and quality and extracted by 1 of the authors. Although we emphasized controlled trials with clinical end points, few such trials exist. Thus, we gave substantial weight to large prospective cohort studies that reported disease outcomes and metabolic studies with established intermediate end points. The evidence is considered strongest when results from different types of studies are consistent.

DIETARY FAT**Metabolic Effects of Dietary Fatty Acids**

Numerous controlled feeding studies of the effects of different dietary fatty acids

on serum cholesterol levels have been summarized in several meta-analyses from which predictive equations have been developed.⁷⁻¹¹ All such analyses confirm early reports by Keys⁷ and Hegsted⁸ that saturated fatty acids increase and polyunsaturated fatty acids decrease total and LDL cholesterol. All 3 classes of fatty acids (saturated, monounsaturated, and polyunsaturated) elevate high-density lipoprotein cholesterol (HDL-C) when they replace carbohydrates in the diet, and this effect is slightly greater with saturated fatty acids (FIGURE 2). Also, triglyceride levels increase when dietary fatty acids are replaced by carbohydrates. Because replacement of saturated fat with carbohydrates proportionally reduces both LDL-C and HDL-C, and, thus, has little effect on the LDL-HDL ratio and increases triglycerides, this change in diet would be expected to have minimal benefit on CHD risk. However, when monounsaturated or polyunsaturated fats replace saturated fat, LDL-C decreases and HDL-C changes only slightly. Moreover, substituting polyunsaturated fat for saturated fat may have beneficial effects on insulin sensitivity^{12,13} and type 2 diabetes.^{14,15}

In numerous controlled metabolic studies, *trans*-fatty acids (found in stick margarine, vegetable shortenings, and commercial bakery and deep-fried foods) have been shown to raise LDL-C levels and lower HDL-C relative to cis-unsaturated fatty acids,¹⁶⁻²⁴ and the increase in the ratio of total to HDL cholesterol for *trans*-fat is approximately twice that for saturated fat (FIGURE 3).²⁵ *Trans*-fat increases plasma levels of lipoprotein a^{18,23} and triglycerides²⁶ and may reduce endothelial function by impairing flow-mediated dilation.²⁷ In addition, *trans*-fatty acids adversely affect essential fatty acid metabolism and prostaglandin balance by inhibiting the enzyme delta-6 desaturase.^{28,29} Finally, high intake of *trans*-fat may promote insulin resistance³⁰ and increase risk of type 2 diabetes.¹⁵

Epidemiologic Studies

Geographic and migration studies showed strong positive correlations be-

OPTIMAL DIETS FOR PREVENTION OF CHD

tween saturated fat intake and rates of CHD.^{43,44} Although these data provide evidence for the importance of environmental factors in the cause of CHD, they are seriously confounded by other aspects of diet, other lifestyle factors, and economic development. Prospective cohort studies of individuals can better control for potential confounding factors. Despite long-standing interest in the diet-heart hypothesis, prospective studies of diet and CHD are surprisingly few;³²⁻⁴⁰ only 2 found a significant positive association between saturated fat intake and risk of CHD.^{35,36} However, most earlier studies were limited by small study size, inadequate dietary assessment, or incomplete adjustment for confounding.⁴¹

The largest and most detailed analysis included 4 repeated measures of diet over 14 years among 80082 women in the Nurses' Health Study cohort.⁴⁰ Higher intakes of *trans*-fat and, to a smaller extent, saturated fat were associated with increased risk, whereas higher intakes of nonhydrogenated polyunsaturated and monounsaturated fats were associated with decreased risk. Because of opposing effects of different types of fat, total fat as percentage of energy was not appreciably associated with CHD risk. Dietary cholesterol and modest egg consumption (1 egg per day) were not significantly associated with either CHD or stroke.⁴²

In addition to the Nurses' Health Study, 3 other large prospective studies have consistently found elevated risk of CHD with higher *trans*-fat intake.^{38,39,43} Combining the results of the 4 prospective studies, the pooled relative risk of CHD associated with a difference of 2% energy in *trans*-fatty acid intake (assessed at baseline) was 1.25 (95% confidence interval, 1.11-1.40).⁴³ Results from case-control studies using biochemical markers of *trans*-fat intake have been less consistent.²⁵ In a recent population-based case-control study of 179 cardiac arrest patients and 285 community controls, higher red-cell membrane levels of *trans*-fatty acids, especially *trans*-isomers from partially hydrogenated

vegetable oils, were associated with significantly increased risk of primary cardiac arrest.⁴⁴ No association was seen in a small UK study of sudden death.⁴⁵

Trials of Change in Dietary Fat

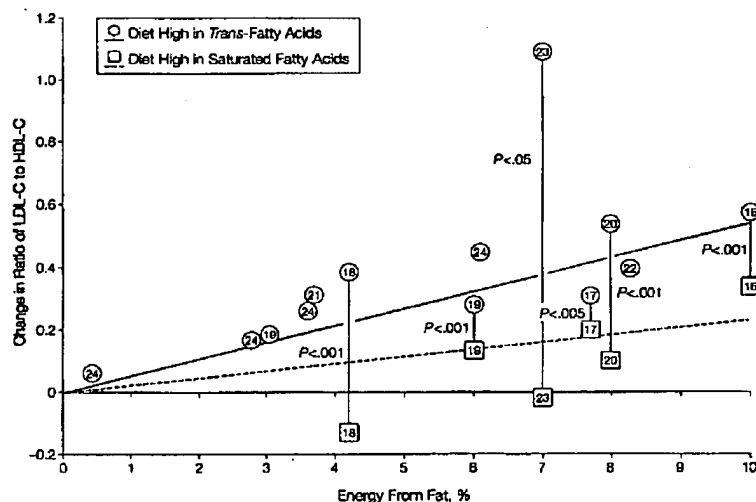
Only a handful of dietary trials with CHD end points have been conducted and most were among patients with CHD (TABLE). Two dietary approaches were tested in earlier trials; one replaced saturated fat with polyunsaturated fat, leaving total fat unchanged; the other lowered total fat. In all the high-polyunsaturated-fat trials,⁴⁶⁻⁵¹ serum cholesterol was significantly reduced. In the Finnish Mental Hospital Study,⁴⁷ soft margarine replaced stick margarine, so the reduction in CHD was probably in part due to reduction in *trans*-fat intake. In the Minnesota Coronary Survey,⁵¹ cardiovascular events were not significantly reduced by a high-polyunsaturated-fat diet despite a decrease in serum cholesterol, but the mean duration of dietary intervention was only about 1 year. Two secondary

prevention trials testing the approach of total fat reduction did not find a significant reduction in serum cholesterol or CHD events.^{52,53}

Omega-3 Fatty Acids

Omega-3 fatty acids may reduce risk of CHD by preventing cardiac arrhythmia, lowering serum triglyceride levels, decreasing thrombotic tendency, and improving endothelial dysfunction.^{54,55} An inverse association between fish intake and coronary mortality was first reported in a Dutch population,⁵⁶ and more than 15 prospective studies have followed. A systematic review of the 11 studies published before 2000 concluded that the inverse association was stronger for fatal CHD than for nonfatal myocardial infarction (MI), and the benefit was most evident in populations with higher-than-average risk of CHD.⁵⁷ Since that review, 4 additional prospective cohort studies⁵⁸⁻⁶¹ and 1 case-control study⁶² have provided further support for the protective effects of marine omega-3 fatty

Figure 3. Results of Metabolic Studies of the Effects of a Diet High in *Trans* or Saturated Fatty Acids on the Ratio of LDL-C to HDL-C



LDL-C indicates low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. A diet with isocaloric amounts of *cis*-fatty acids was used as the comparison group. References are indicated by numbers inside data markers. The solid line indicates the best-fit regression for *trans*-fatty acids. The dashed line indicates the best-fit regression for saturated fatty acids. Reprinted with permission.⁵⁸

OPTIMAL DIETS FOR PREVENTION OF CHD

acids against CHD in diverse populations. Notably, 2 recent studies have shown that consuming 2 or more servings of fish per week was associated with 30% lower risk of CHD in women⁶⁰ and that blood levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were strongly associated with decreased risk of sudden cardiac death in men.⁶¹

α -Linolenic acid (ALA), an omega-3 fatty acid high in flaxseed, canola, and soybean oils, can be converted to EPA

and DHA in humans and, thus, may have a role in prevention of CHD. An inverse association between intake of ALA and risk of fatal CHD was observed in most prospective cohort studies,^{38,39,63,64} but not in 1 smaller study.⁶⁵ In a cohort of women, frequent consumption of oil-and-vinegar salad dressing (a major source of ALA in US diets) was associated with a significantly lower risk of fatal CHD.⁶³

Three clinical trials have examined the effects of omega-3 fatty acids in sec-

ondary prevention of CHD (Table). In the Diet and Reinfarction Trial,⁵³ patients advised to eat fish twice weekly or to take fish oil (1.5 g/d) had a 29% lower mortality after 2 years. In the GISSI-Prevenzione trial,⁶⁶ daily supplementation with EPA plus DHA (1 g/d) reduced the main end point (composite of death, nonfatal MI, and stroke) by 15%, primarily because of a 45% reduction in sudden death after 3 months of treatment.⁶⁷ A trial from India suggested benefits of both fish oil and mus-

Table. Trials of Dietary Interventions and Coronary Events*

Trial	Patients in Intervention Group	Dietary Intervention	Dietary Fat (Energy) in Treatment Group, %	Energy From P and S Fat in Treatment Group, %	Overall Trial Duration, y	Change in Serum Cholesterol Level, %†	Change in CHD, %‡
Low-Fat Approach							
MRC (low fat) ⁵²	123 male MI patients	Reduce total fat	22	NR	3	-5	+4
DART ⁵³	1015 male MI patients	Reduce total fat	32	NR	2	-4	-9
High-Polyunsaturated-Fat Approach							
Finnish Mental Hospital Study ⁴⁷	676 men without CHD	Reduce saturated fat, increase polyunsaturated fat	35	P = 13; S = 9	6	-15	-44§
Los Angeles Veteran Study ⁴⁸	424 men; most had no evidence of existing CHD	Reduce saturated fat, increase polyunsaturated fat	40	P = 16; S = 9	8	-13§	-20 in CHD, -31§ in cardiovascular events
Oslo Diet-Heart Study ^{48,49}	206 male MI patients	Reduce saturated fat, increase polyunsaturated fat	39	P = 21; S = 9	5	-14§	-25§
MRC (soy oil) ⁵⁰	199 male MI patients	Reduce saturated fat, increase polyunsaturated fat	46	P:S ratio = 2	4	-15§	-12
Minnesota Coronary Survey ⁵¹	4383 men and 4664 women	Reduce saturated fat, increase polyunsaturated fat	38	P = 15; S = 9	1	-14§	0
Increase Omega-3 Fatty Acid							
DART ⁵³	1015 male MI patients	Fish twice per week or fish oil (1.5 g/d)	NR	NR	2	NR	-16 in CHD events, -29§ in total mortality
GISSI-Prevenzione ^{66,67}	5666 MI patients, primarily men	Fish oil (EPA + DHA, 1 g/d)	NR	NR	3.5	0	-30§ in cardiovascular death, -45§ in sudden death
Indian Experiment of Infarct Survival 4 ⁵⁸	242 MI patients, primarily men	Fish oil (EPA, 1.08 g/d) or mustard oil (ALA, 2.9 g/d)	NR	NR	1	0	-30§ in fish oil group, -19 in mustard oil group
Whole-Diet Approach							
Lyon Diet Heart Study ^{59,60}	302 MI patients, primarily men	High ALA intake and Mediterranean diet	31	P:S ratio = 0.7	3.8	0	-72§
Indian Experiment of Infarct Survival ¹¹⁷	204 MI patients, primarily men	High intake of fruits, vegetables, nuts, fish, and pulses	24	P:S ratio = 1.2	1	-9§	-40§

*Adapted from Hu et al.¹²⁶ P indicates polyunsaturated fat; S, saturated fat; CHD, coronary heart disease; MRC, Medical Research Council; MI, myocardial infarction; NR, not reported; DART, Diet and Reinfarction Trial; GISSI, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; and ALA, α -linolenic acid.

†Change in cholesterol level refers to the percentage change in serum cholesterol level in the treatment group compared with the change in the control group.

‡Change in CHD refers to the percentage difference in coronary event rates in the treatment group compared with the control group.

§P < .05.

||The total duration of the study was 4.5 years, but the mean duration of the intervention was only 1 year.

OPTIMAL DIETS FOR PREVENTION OF CHD

tard oil in the treatment of MI patients.⁶⁸ In the Lyon Diet Heart Study, higher ALA consumption in the context of a Mediterranean diet dramatically reduced total and cardiovascular mortality as well as nonfatal MI.^{69,70} These trials strongly support the protective effects of omega-3 fatty acids, including both ALA and fish oil, in secondary prevention of CHD. The role of fish oil supplements in primary prevention of CHD has not been tested.

CARBOHYDRATES

Prevailing dietary recommendations have emphasized high intake of complex carbohydrates, mainly starch, and avoidance of simple sugars.^{71,72} However, many starchy foods, such as baked potatoes and white bread, are rapidly digested to glucose and produce even higher glycemic and insulinemic responses than sucrose (half glucose and half fructose). The glycemic index (GI) ranks foods based on rise in blood glucose (the incremental area under the curve for blood glucose levels) after ingestion compared with glucose or white bread, standardizing the carbohydrate content to 50 g.^{73,74} Foods with a low degree of starch gelatinization (more compact granules), such as spaghetti and oatmeal, and a high level of viscous soluble fiber, such as barley, oats, and rye, tend to have a slower rate of digestion and, thus, lower GI values. In several controlled clinical studies,⁷⁵ feeding low-GI meals to diabetic patients led to significant improvement in glycemic control and lipid profile, but larger studies are needed.

Glycemic load (GL; the product of the GI value of a food and its carbohydrate content) has been used to represent both the quality and quantity of the carbohydrates consumed.^{76,77} Dietary GL is more strongly associated with higher fasting triglycerides and lower HDL-C levels compared with GI.⁷⁸ A strong positive association between GL and risk of CHD was observed among 75521 women during 10 years of follow-up.⁷⁹ The increased risk was more pronounced among overweight and obese women, consistent with meta-

bolic studies that the adverse effects of a high GL diet are exacerbated by underlying insulin resistance.⁸⁰ Thus, carbohydrate-containing foods should not be judged simply by their GI values; the amount of carbohydrates, fiber, and other nutrients are also important.

Another way to classify dietary carbohydrates is to subdivide cereal grains—staple foods in most societies—into whole and refined grains. Most cereal grains are highly processed before they are consumed. Refined grain products contain more starch but substantially lower amounts of dietary fiber, essential fatty acids, and phytochemicals, although these products are typically enriched with some vitamins and minerals. In several epidemiologic studies, higher consumption of whole grains was associated with lower risk of CHD. Also, prospective cohort studies have consistently found an inverse association between fiber intake and risk of CHD.⁸¹ Several studies have found a stronger association for cereal fiber than for fruit or vegetable fiber.⁸²⁻⁸⁴ The inverse association for fiber observed in epidemiologic studies cannot be fully explained by its cholesterol-lowering effects; the low GI of foods with a high level of fiber and numerous micronutrients in whole grains may also contribute to the benefits.⁸⁵

FOLATE

Much evidence suggests that adequate folate consumption is important for the prevention of CHD. Epidemiologic studies have found an inverse association between folate intake measured by dietary questionnaire or serum folate level and risk of CHD,⁸⁶⁻⁹⁰ which is likely to be mediated through homocysteine-lowering effects of folic acid. Two randomized placebo-controlled trials evaluated effects of folic acid supplementation on the development and progression of atherosclerosis. Vermeulen et al⁹¹ found that supplementation with folic acid and vitamin B₆ for 2 years significantly decreased subclinical atherosclerosis indicated by abnormal exercise electrocardiography tests among siblings of patients with existing car-

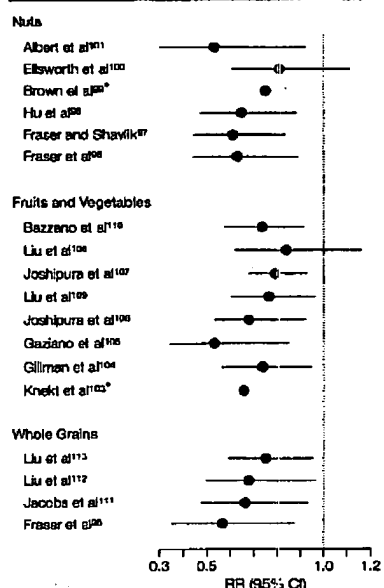
diovascular disease. In the Swiss Heart Study, treatment with a combination of folic acid and vitamins B₆ and B₁₂ significantly decreased restenosis and revascularization after coronary angioplasty at 6 months⁹² and a combined cardiovascular end point at 11 months.⁹³ Ongoing clinical trials should provide more definitive data on the role of folic acid supplementation in CHD prevention, but the interpretation of the findings from trials conducted in the United States could be complicated by the fortification of flour with folic acid.⁹⁴

SPECIFIC FOODS AND DIETARY PATTERNS

The relationship between consumption of specific foods or overall dietary patterns and risk of CHD has been examined in recent studies. Such analyses are valuable in evaluating additional diet-heart hypotheses and in making practical dietary recommendations. For example, replacement of red meat with chicken and fish has been associated with reduced risk of CHD.⁹⁵ An inverse association between nut consumption and risk of CHD has been seen consistently in prospective studies.⁹⁶⁻¹⁰¹ (FIGURE 4), which further underscores the importance of distinguishing different types of fat. Although nuts are high in fat and, thus, routinely proscribed in dietary recommendations, the predominant types of fat in nuts are monounsaturated and polyunsaturated, which lower LDL-C level.¹⁰²

Although beneficial effects of fruits and vegetables are widely assumed, only in recent years has solid epidemiologic evidence begun to emerge¹⁰³⁻¹¹⁰ (Figure 4). In the largest study, including 84251 women and 42148 men,¹⁰⁷ Joshipura et al reported a significant inverse association between consumption of fruits and vegetables, particularly green leafy vegetables and vitamin C-rich fruits and vegetables, and risk of CHD. Increased consumption of potatoes, however, was not associated with benefits. In several prospective studies, a higher consumption of whole grains as opposed to refined grains was

OPTIMAL DIETS FOR PREVENTION OF CHD

Figure 4. Prospective Cohort Studies of Cardiovascular Disease and Consumption of Nuts, Fruits and Vegetables, or Whole Grains

Relative risks (RRs) and 95% confidence intervals (CIs) were derived from the comparison of the incidence rates between the highest- and lowest-consumption groups (quintiles, quartiles, or specific intake categories) and were adjusted for nondietary and/or dietary covariates. In each category, studies are shown in the order of most recent to least recent publication. Asterisk indicates confidence intervals were not published in the original article.

associated with a lower risk of cardiovascular disease (Figure 4).^{96,111-113}

Recently, several studies have reported the role of overall dietary patterns in predicting long-term risk of CHD.¹¹⁴ In these analyses, a "prudent" pattern characterized by higher intakes of fruits, vegetables, legumes, whole grains, poultry, and fish was associated with lower risk of CHD, whereas a "Western" pattern characterized by higher intakes of red and processed meats, sweets and desserts, potatoes, french fries, and refined grains was associated with a higher risk, independent of lifestyle factors.^{115,116}

Two randomized trials tested the whole-diet approach in secondary prevention of CHD (Table). In the Indian Heart Study,¹¹⁷ a semivegetarian diet en-

riched with fruits, vegetables, whole grains, and nuts reduced coronary death by 41% and nonfatal MI by 38%. In the Lyon Diet Heart Study,^{69,70} a Mediterranean diet enriched with ALA reduced CHD death by more than 70%. These findings, together with the results from prospective cohort studies and the Dietary Approaches to Stop Hypertension trials,^{118,119} support the clinical utility of a whole-diet approach in the prevention of cardiovascular disease.

COMBINED EFFECTS OF DIET AND LIFESTYLE

The combination of multiple dietary factors is more powerful than a single factor alone. In the Nurses' Health Study cohort, a diet high in cereal fiber, marine omega-3 fatty acids, and folate and low in *trans*-fat and GL, with a high ratio of polyunsaturated fat to saturated fat, strongly predicted decreased risk of CHD (relative risk comparing highest with lowest quintiles of the composite score=0.40; 95% confidence interval, 0.31-0.53).¹²⁰ Also, improvement in these dietary factors explained much of the decline in the incidence of CHD during 14 years of follow-up of the cohort.¹²¹

Besides diet, several other behavioral factors strongly influence CHD risk. Analyses from the Nurses' Health Study estimated that 82% of CHD events in the study cohort could be potentially prevented by moderate diet and lifestyle modifications.¹²⁰ Among nonsmokers, 74% of coronary events might have been prevented by eating a healthy diet, maintaining a healthy body weight, exercising regularly for half an hour or more daily, and consuming a moderate amount of alcohol (≥ 5 g/d).

Results from several multifactorial primary prevention trials using diet and lifestyle intervention have been largely unimpressive, probably because of poor compliance and inadequate power.¹²² The Oslo Heart Study, however, demonstrated that stopping smoking and increasing the ratio of polyunsaturated to saturated fats in the diet reduced CHD incidence by 47% among men with

higher-than-average serum cholesterol levels.¹²³ In the Lifestyle Heart Study,¹²⁴ a combination of an extremely low-fat diet, exercise, stress management, and yoga significantly reduced progression of atherosclerosis, but the low-fat regimen is unnecessarily rigid and difficult for most people to follow.

AREAS OF UNCERTAINTY

The optimal amounts of monounsaturated and polyunsaturated fats in the diet are still unclear. Intake of linoleic acid is usually recommended not to exceed 10% of energy, in part because of little long-term human experience with such diets, although benefits from higher intake exist for blood lipids. There has been some concern that a high-polyunsaturated-fat diet may increase cancer risk, but this has not been substantiated in large epidemiologic studies.¹²⁵

The optimal balance between omega-3 and omega-6 polyunsaturated fatty acids also remains unsettled. Some have proposed reducing the consumption of linoleic acid to achieve a greater ratio of omega-3 to omega-6 fatty acids in the diet.^{126,127} However, there is little evidence that a higher ratio predicts a lower risk of CHD.¹²⁸ Both omega-3 and omega-6 fatty acids have important roles in reducing CHD risk, probably through different mechanisms. Thus, a good strategy is to substantially increase intake of omega-3 fatty acids from fish and plant sources (because intake for many people is clearly suboptimal) without decreasing intake of linoleic acid. This will improve the ratio and maximize the cardioprotective benefits of both omega-3 and omega-6 fatty acids.

The amount and type of protein in the diet is a matter of debate. Substitution of soy for animal protein reduces LDL-C,¹²⁹ and substituting animal protein for carbohydrates raises HDL-C and lowers triglyceride levels.¹³⁰ Consistent with the metabolic studies, a prospective cohort study found that a moderately high protein intake (24% vs 15% of energy from protein) was associated with a sig-

OPTIMAL DIETS FOR PREVENTION OF CHD

nificantly lower risk of CHD after adjustment for cardiovascular risk factors and dietary fat intake.¹³¹ To avoid an increase in saturated fat intake, the major source of protein in the diet should come from nuts, soybeans, legumes, poultry, and fish.

The role of phytochemicals and antioxidants in the prevention of CHD is promising but unsettled. The cholesterol-lowering effects of plant sterol or stanol (saturated sterols) have been well documented in clinical trials¹³² and commercial products made of these compounds are widely available, but their long-term effects remain to be seen. Six prospective cohort studies have evaluated the association between flavonoid intake and risk of CHD. A significant inverse association was observed in some studies¹³³⁻¹³⁶ but not others.^{137,138} Although a body of experimental evidence has demonstrated the role of antioxidant vitamins in reducing oxidative stress and substantial epidemiologic evidence has linked intake of vitamin E with a lower CHD risk, results from published clinical trials of vitamin E supplements, primarily among patients with clinical CHD, have been largely disappointing.^{139,140} Ongoing primary prevention trials should provide more insights.

Finally, a large and inconclusive literature has examined the relationship between dietary minerals such as calcium, magnesium, zinc, and selenium and risk of CHD.⁴¹ Most studies have been based on ecological correlations or case-control analyses. Additional large prospective studies or randomized trials with clinical end points are required to resolve the role of individual minerals from diet or supplements.

CONCLUSIONS

Compelling evidence from metabolic studies, epidemiologic investigations, and clinical trials in the past several decades converges to indicate that at least 3 dietary strategies are effective in preventing CHD: substitute unsaturated fats (especially polyunsaturated fat) for saturated and *trans*-fats; increase con-

sumption of omega-3 fatty acids from fish oil or plant sources; and consume a diet high in fruits, vegetables, nuts, and whole grains and low in refined grains. A combination of these approaches can confer greater benefits than a single approach. However, simply lowering the percentage of energy from total fat in the diet is unlikely to improve lipid profile or reduce CHD incidence.

Obesity is an important avenue by which diet can influence risk of CHD. However, the relationship between diet, especially dietary fat, and obesity remains controversial. Although reduction in percentage of calories from dietary fat intake is commonly recommended for weight loss, long-term clinical trials have provided no good evidence that reducing dietary fat per se can lead to weight loss.^{141,142} There is a growing consensus that excess calories, whether from carbohydrates or fat, will induce weight gain. A mildly hypocaloric moderate-fat diet, which allows for a great variety in choosing foods, can have better long-term compliance than a typical low-fat diet.¹⁴³ Small short-term studies have suggested roles of several diets in weight control, including a low-GI diet,¹⁴⁴ a high-protein diet,^{145,146} and a diet high in dairy products,¹⁴⁷ but larger and long-term studies are needed.

Although prevailing dietary guidelines emphasize target intake of specific macronutrients (eg, not exceeding 30% of energy from fat),⁷¹ such numerical criteria are not based on solid scientific evidence, and the public finds it difficult to make dietary changes based on such criteria. A variety of options exist for designing attractive and heart-healthy diets, with varying amounts of fat and carbohydrates, as long as the diet embraces healthy types of fat and carbohydrates and provides an appropriate balance in energy intake and expenditure. Evidence is now clear that diets including nonhydrogenated unsaturated fats as the predominant form of dietary fat, whole grains as the main form of carbohydrate, an abundance of fruits and vegetables, and

adequate omega-3 fatty acids can offer significant protection against CHD. Such diets, together with regular physical activity, avoidance of smoking, and maintaining a healthy weight, may prevent the majority of cardiovascular disease in Western populations.

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OPTIMAL DIETS FOR PREVENTION OF CHD

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To spend too much time in studies is sloth; to use them too much for ornament, is affectation; to make judgment wholly by their rules, is the humour of a scholar. . . . Read not to contradict and confute; nor to believe and take for granted; nor to find talk and discourse; but to weigh and consider. Some books are to be tasted, others to be swallowed, and some few to be chewed and digested. . . . Reading maketh a full man; conference a ready man; and writing an exact man. . . . Histories make men wise; poets witty; the mathematics subtle; natural philosophy deep; moral grave; logic and rhetoric able to contend.

—Francis Bacon (1561-1626)

High-Fat Diet-Induced Hyperglycemia and Obesity in Mice: Differential Effects of Dietary Oils

Shinji Ikemoto, Mayumi Takahashi, Nobuyo Tsunoda, Kayo Maruyama, Hiroshige Itakura, and Osamu Ezaki

Mice fed a high-fat diet develop hyperglycemia and obesity. Using non-insulin-dependent diabetes mellitus (NIDDM) model mice, we investigated the effects of seven different dietary oils on glucose metabolism: palm oil, which contains mainly 45% palmitic acid (16:0) and 40% oleic acid (18:1); lard oil, 24% palmitic and 44% oleic acid; rapeseed oil, 59% oleic and 20% linoleic acid (18:2); soybean oil, 24% oleic and 54% linoleic acid; safflower oil, 76% linoleic acid; perilla oil, 58% α -linolenic acid; and tuna fish oil, 7% eicosapentaenoic acid and 23% docosahexaenoic acid. C57BL/6J mice received each as a high-fat diet (80% of total calories) for 19 weeks ($n = 6$ to 11 per group). After 19 weeks of feeding, body weight induced by the diets was in the following order: soybean > palm \approx lard \approx rapeseed \approx safflower \approx perilla > fish oil. Glucose levels 30 minutes after a glucose load were highest for safflower oil (≈ 21.5 mmol/L), modest for rapeseed oil, soybean oil, and lard (≈ 17.8 mmol/L), mild for perilla, fish, and palm oil (≈ 13.8 mmol/L), and minimal for high-carbohydrate meals (≈ 10.4 mmol/L). Only palm oil-fed mice showed fasting hyperinsulinemia ($P < .001$). By stepwise multiple regression analysis, body weight (or white adipose tissue [WAT] weight) and intake of linoleic acid (or n-3/n-6 ratio) were chosen as independent variables to affect glucose tolerance. By univariate analysis, the linoleic acid intake had a positive correlation with blood glucose level ($r = .83$, $P = .02$) but not with obesity ($r = .46$, $P = .30$). These data indicate that (1) fasting blood insulin levels vary among fat subtypes, and a higher fasting blood insulin level in palm oil-fed mice may explain their better glycemic control irrespective of their marked obesity; (2) a favorable glucose response induced by fish oil feeding may be mediated by a decrease of body weight; and (3) obesity and a higher intake of linoleic acid are independent risk factors for dysregulation of glucose tolerance.

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IT IS WIDELY ACCEPTED that non-insulin-dependent diabetes mellitus (NIDDM) is caused by a combination of genetic and environmental factors, notably diet and level of physical activity.^{1,3} Among the environmental factors, the high-fat content of the typical Western diet is considered a major cause of obesity-associated insulin resistance.^{4,6} Epidemiological^{4,5} and animal⁶ studies made on several environmental factors suggest that the high fat content of the typical Western diet is a major cause of obesity and insulin resistance. Indeed, various metabolic studies have confirmed the view that calories obtained from fat have a greater effect on obesity than energy per se.^{7,8} Among several dietary oils, monounsaturated oil^{9,10} and fish oil¹¹ have been proposed as favorable diets for NIDDM patients. In rats made insulin-resistant with a high-fat diet, the resistance can be prevented by the addition of fish oil,¹² with improvement of insulin resistance and, incorporation of highly unsaturated long-chain n-3 fatty acids into the phospholipid component of muscle tissues.¹³ Borkman et al¹⁴ have shown that in patients with coronary artery disease, fasting serum insulin concentration (a marker of insulin resistance) is correlated positively with the percentage of 18:2 (linoleic acid) in the phospholipid fraction of muscle, but negatively with individual long-chain polyunsaturated fatty acids such as 20:4 (arachidonic acid), 22:4 (n-6), 22:5 (n-6), and 22:5 (n-3). However, clinical trials using fish oil supplements failed to show a marked improvement in glucose control.¹⁵⁻¹⁸ These data indicate that there are some discrepancies between the insulin resistance at tissue level and the actual blood glucose level. The importance of fatty acid composition of dietary oils seems evident, but it has been difficult to select favorable oils from human studies because of the difficulty involved in long-term study of dietary oil intake. Indeed, we do not know whether a higher intake of linoleic acid or a lower intake of fish oil (or n-3) is responsible for the development of insulin resistance.

Feeding a high-fat diet in certain strains of mice provides

a suitable model of NIDDM^{19,20} and atherosclerosis.²¹ The present studies were designed to clarify the differences in the effects of long-term feeding of commercially available dietary oils on glucose metabolism and obesity using this NIDDM mouse model. In comparison with high-carbohydrate feeding, the effects of seven dietary oils were investigated: palm oil, which contains mainly 45% palmitic acid (16:0) and 40% oleic acid (18:1); lard oil, 24% palmitic and 44% oleic acid; rapeseed oil, 59% oleic and 20% linoleic acid (18:2); soybean oil, 24% oleic and 54% linoleic acid; safflower oil, 76% linoleic acid; perilla oil, 58% α -linolenic acid; and tuna fish oil, 7% eicosapentaenoic acid and 23% docosahexaenoic acid.

MATERIALS AND METHODS

Animals

C57BL/6J female mice were obtained from Tokyo Laboratory Animals Science (Tokyo, Japan) at 7 weeks of age and fed a high-carbohydrate diet (Table 1) for 1 week to accommodate to a new environment. The mice were maintained at a constant temperature of 22°C with a fixed artificial light cycle (12 hours light and 12

From the Division of Clinical Nutrition, National Institute of Health and Nutrition, Tokyo, Japan.

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Address reprint requests to Osamu Ezaki, MD, Division of Clinical Nutrition, National Institute of Health and Nutrition, 1-23-1, Toyama, Shinjuku-ku, Tokyo 162, Japan.

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1540

IKEMOTO ET AL

Table 1. Composition of the High-Carbohydrate Diet and High-Fat Diets

Component	High-Carbohydrate (%)	High-Fat (%)
Oil	4.0	32.0
Casein	23.7	33.1
Sucrose	10.3	17.6
α -Starch	50.0	—
Vitamin mix	1.0	1.4
Mineral mix	7.0	9.8
Cellulose powder	4.0	5.6
DL-Methionine	0.4	0.5
Energy (kcal/100 g)	343.4	469.8
Fat energy kcal/100 g	36.8	294.7
%	10.7	60.2

hours dark). The mice were allowed free access to either a high-carbohydrate diet or various high-fat diets.

Diet

The composition of the high-carbohydrate diet and high-fat diets is shown in Table 1. Fatty acid compositions of dietary oils were measured by gas-liquid chromatography and are shown in Table 2. In the high-carbohydrate diet, safflower oil was used as source of fat. Every 2 weeks, ingredients for the purified diets were mixed, formed into a dough with water, rolled into pellets, wrapped with plastic wrap, and stored at -20°C until use to minimize oxidation. These small pellets were given to mice every day. Preliminary feeding trials were conducted, and the composition of the diets was adjusted so that the daily intake of calories and the amount of dietary components except fat and carbohydrate were nearly identical. Casein, sucrose, starch, vitamin mixture, mineral mixture, and cellulose powder were purchased from Oriental Yeast (Tokyo, Japan); palm oil and soybean oil from Fuji Oil (Osaka, Japan); lard oil from Snow Brand Milk Products (Tokyo, Japan); rapeseed oil from Yonezawa Oil (Saitama, Japan); safflower oil from Benibana Food (Tokyo, Japan); perilla oil from Ohta Oil (Aichi, Japan); fish oil from NOF (Tokyo, Japan); and DL-methionine from Sigma (St Louis, MO).

Experimental Procedures

Mice were divided into eight groups. The first group was given the high-carbohydrate diet, which on a caloric basis consisted of 63% carbohydrate, 11% fat, and 26% protein. The other seven groups were given various high-fat diets containing 14% carbohydrate, 60% (of several types of) fat, and 26% protein (Table 1). Mice fed each diet for 16 weeks were fasted overnight, and then blood samples were obtained by snipping the tail for insulin, triglyceride, and cholesterol assays. Mice were killed at 19 weeks of feeding, and body weight, body weight gain, and wet white adipose tissue (WAT) weight were measured. Oral glucose tolerance tests were also conducted at 3 and 18 weeks of feeding. After killing the mice with an intraperitoneal injection of pentobarbital (Abbott, North Chicago, IL) 0.05 mg/g body weight, parametrial white adipose tissue and gastrocnemius were excised for measurement of weight and GLUT4 protein level, respectively.

Food Intake Measurements

The number of mice per cage was six to eight. For high-carbohydrate or high-safflower oil feeding, two cages were used; for other oil feeding, one was used. For food intake measurements at 14 weeks of feeding, mice that had been kept in plastic shoe box-type cages with paper chips (Alpha Dri; Shepherd Specialty Papers, Kalamazoo, MI) were transferred to shoe box cages with wire bottoms. Beneath the wire, newspapers were spread out to collect food spillage. After removing feces on the paper, food spillage on the paper was collected and dried in an oven to evaporate water originating from urine. To accommodate mice to cages with wire bottoms, food intake measurements were started 2 days after transferring them to new cages. Food intake measurement was made every day for 5 days, and then mice were returned to the cages with paper chips. The mean food intake per day was estimated by subtracting the weight of food spillage from the initial food weight (dry form) in the cage and dividing by the number of mice housed in the cage. Thus, the standard error for food intake shown in Table 3 was from the variation of daily intake, but not from that of the individual mouse.

Oral Glucose Tolerance Test

Eighteen weeks after feeding the experimental diets, D-glucose (1 mg/g body weight) was administered after an overnight fast, by stomach tube. Blood samples were obtained by snipping the tail

Table 2. Fatty Acid Composition (%) of the Dietary Oils

Fatty Acid	Palm	Lard	Rapeseed	Soybean	Safflower	Perilla	Fish
12:0	0.4						3.0
14:0	1.1	1.7				6.1	18.2
16:0	44.5	24.0	3.8	9.9	6.8		4.2
16:1	0.2	2.8	0.2			1.7	4.9
18:0	4.2	14.4	1.7	4.1	2.5	18.4	18.8
18:1	39.5	43.9	59.4	23.6	13.8	14.3	1.3
18:2 (n-6)	9.2	9.1	20.2	53.7	75.7	58.3	0.8
18:3 (n-3)	0.2	0.7	7.1	7.1	0.2		2.0
20:4 (n-6)		0.1					6.8
20:5 (n-3)							22.8
22:6 (n-3)						1.2	17.2
Others	0.7	3.5	7.6	1.6	1.0		
S:M:P ratio	10:8:2	10:12:2	10:95:41	10:16:42	10:14:76	10:24:92	10:10:14
n-6/n-3 ratio	46.1	13.1	2.9	7.6	378.5	0.3	0.1

Abbreviation: S:M:P, saturated, monounsaturated, and polyunsaturated fatty acid.

DIETARY OILS ON OBESITY AND HYPERGLYCEMIA

1541

Table 3. Food Intake, Final Body Weight, Body Weight Gain, Parametrial WAT Weight, and Fasting Insulin, Triglyceride, and Cholesterol Levels

	High-Carbohydrate (n = 11)	High-Fat							ANOVA
		Palm (n = 6)	Lard (n = 8)	Rapeseed (n = 6)	Soybean (n = 6)	Safflower (n = 8)	Perilla (n = 5-8)	Fish (n = 7)	
Food intake (kcal/mouse/d)	7.3 ± 0.5	7.5 ± 0.5	9.1 ± 0.2	7.1 ± 0.3	8.1 ± 0.3	8.6 ± 0.8	6.4 ± 0.4	9.8 ± 0.4†	F(7,44) = 2.9 P = .013
Final body weight (g)	26.8 ± 0.9	40.2 ± 2.2†	40.2 ± 2.4†	38.8 ± 1.4†	45.5 ± 2.7†	38.2 ± 2.1†	33.4 ± 2.8*	25.9 ± 1.7	F(7,50) = 12.8 P < .0001
Body weight gain (g)	9.5 ± 0.8	23.0 ± 1.9†	23.1 ± 2.3†	21.6 ± 1.5†	28.3 ± 2.9†	20.9 ± 2.0†	16.3 ± 2.5*	9.0 ± 1.6	F(7,50) = 13.9 P < .0001
WAT weight (g)	0.84 ± 0.10	2.47 ± 0.20†	2.73 ± 0.26†	3.03 ± 0.25†	4.14 ± 0.43†	2.52 ± 0.21†	1.95 ± 0.40†	0.91 ± 0.22	F(7,50) = 20.4 P < .0001
Insulin (pmol/L)	84.8 ± 17.9	245.0 ± 51.5†	93.6 ± 10.3	112.9 ± 27.5	87.2 ± 17.6	107.1 ± 16.5	112.9 ± 22.5	43.1 ± 4.0	F(7,51) = 2.7 P = .020
Triglyceride (mg/dL)	71.0 ± 6.8	67.6 ± 11.5	51.9 ± 3.3*	40.5 ± 7.2†	42.1 ± 2.2†	35.1 ± 3.7†	41.6 ± 4.9†	35.9 ± 4.1†	F(7,51) = 6.0 P < .0001
Total cholesterol (mg/dL)	77.5 ± 3.3	96.8 ± 4.1†	92.8 ± 2.2†	68.6 ± 2.5*	74.0 ± 3.5	75.0 ± 3.1	70.4 ± 3.5	73.2 ± 2.7	F(7,51) = 9.6 P < .0001

NOTE. Results are the mean ± SE of individual mean values obtained in each of 5 to 11 mice. Food intakes were measured for 5 days and are expressed as the mean ± SE intake per day.

*P < .05, †P < .01, ‡P < .001: high-carbohydrate v other groups by Fisher's protected least-significant difference test.

before and at 30, 60, and 120 minutes after glucose administration. Blood glucose levels were measured using a TIDEX glucose analyzer (Sankyo, Tokyo, Japan).

Immunoblotting

Crude membrane fractions from skeletal muscle (gastrocnemius) were prepared as described previously.²² Proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were electrophoretically transferred to Immobilon (Millipore, Bedford, MA) and immunoblotted with antibodies directed against the C-terminal amino acid sequence of GLUT4 and then ¹²⁵I-labeled protein A (ICN, Costa Mesa, CA) as described previously.²² The amount of protein used per gel lane was 60 µg. The amount of GLUT4 was quantified with an image analyzer (BAS 2000; Fuji Film, Tokyo, Japan).

Other Analyses and Methods

Immunoreactive insulin level was measured by radioimmunoassay (RIA) using a rat insulin RIA kit (Inestar, Stillwater, MN). Triglyceride and total cholesterol levels were measured by enzyme assays, determiner LTG and TCS55, respectively (Kyowa Medics, Tokyo, Japan). Protein was assayed using the Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, IL).

Statistical Analysis

Statistical comparisons of the groups were made by ANOVA, and each group was compared with the others by Fisher's protected least-significant difference test (Statview 4.0, Abacus Concepts, Berkeley, CA). The glucose tolerance curve of each group was compared by repeated-measures ANOVA (Super ANOVA; Abacus Concepts). Relations between variables were analyzed by a simple correlation and a stepwise multiple regression model (Statview 4.0; Abacus Concepts). Statistical significance is defined as P less than .05; values are the mean ± SE.

RESULTS

After 19 weeks' feeding, body weight obtained with the diets was in the following order: soybean oil > palm oil ≥ lard oil ≥ rapeseed oil ≥ safflower oil > perilla oil > high-carbohydrate diet ≥ fish oil (Table 3). Mice fed a

high-soybean oil diet showed a 70% increase in body weight compared with mice fed a high-carbohydrate diet. Parallel to the body weight change, the wet weight of parametrial WAT was in order of soybean oil > rapeseed oil ≥ lard oil ≥ safflower oil ≥ palm oil > perilla oil > fish oil ≥ high-carbohydrate diet (Table 3). Indeed, the correlation between body weight and WAT weight was high ($r = .95$, $P < .0001$, $n = 58$). It should be noted that mice were allowed free access to food. Since the intake of high-fat diet mice was less than that of high-carbohydrate diet mice, the total calorie intake of both groups became nearly identical. However, the energy intake for fish oil was significantly higher than for carbohydrate or palm, rapeseed, and perilla oils, but the level for perilla oil was less than for lard or safflower and fish oils (Table 3). Other values were not significant.

After 3 weeks' feeding, all high-fat diets resulted in significant increase of blood glucose levels 30, 60, and 120 minutes after an oral glucose challenge compared with the high-carbohydrate diet (Fig 1). However, after 18 weeks of feeding, each dietary oil showed a different response to the glucose challenge. Glucose levels 30 minutes after the glucose load were highest for safflower oil (≈ 21.5 mmol/L), modest for rapeseed oil, soybean oil, and lard (≈ 17.6 mmol/L), mild for perilla oil, fish oil, and palm oil (≈ 13.8 mmol/L), and minimal for a high-carbohydrate diet (≈ 10.4 mmol/L). Significance was established by repeated-measures ANOVA ($P < .001$, carbohydrate v lard oil, rapeseed oil, and safflower oil; $P < .01$, carbohydrate v soybean oil; $P < .01$, safflower oil v fish oil and palm oil; $P < .05$, carbohydrate v perilla oil and fish oil; and $P < .05$, safflower oil v lard and perilla oil). In these oral glucose tolerance tests, obese mice received a higher amount of glucose than lean mice, since the amount of glucose given orally was determined on a body-weight basis. However, the contribution of different amounts of glucose to the glucose tolerance curve in each group of mice was minimal, since in the

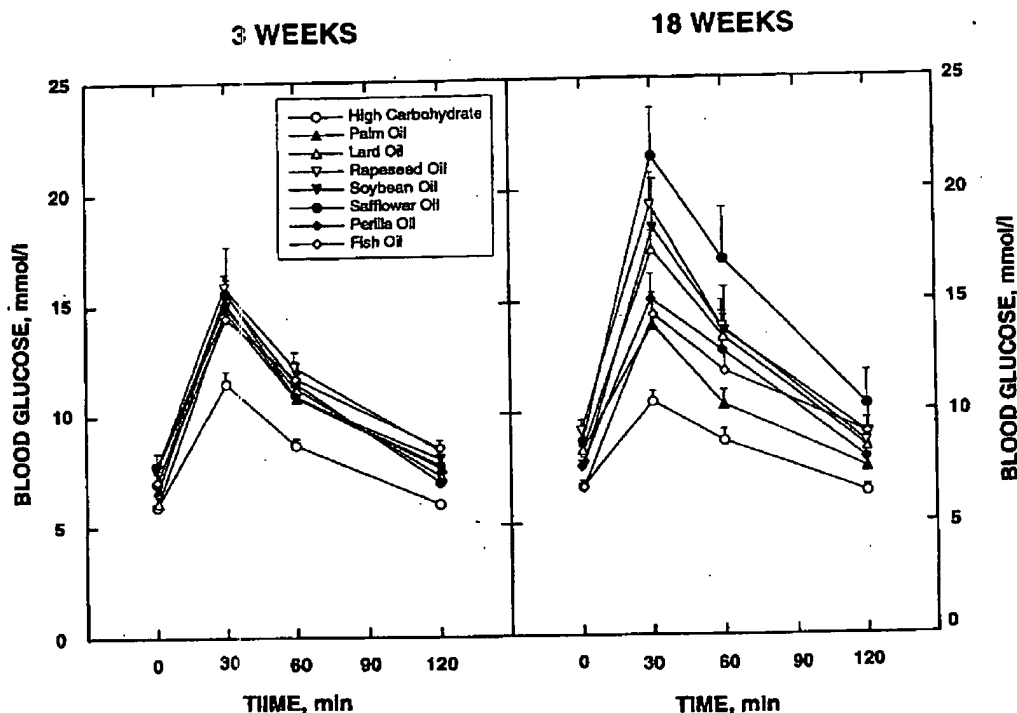


Fig 1. Oral glucose tolerance tests. Each data point represents the mean of 3 to 7 mice at 3 weeks and 4 to 10 mice at 18 weeks. At 3 weeks of feeding: $P < .001$, carbohydrate v palm oil, lard oil, rapeseed oil, soybean oil, safflower oil, perilla oil, and fish oil; $P < .05$, lard oil v rapeseed oil. At 18 weeks of feeding, $P < .001$, carbohydrate v lard oil, rapeseed oil, and safflower oil; $P < .01$, carbohydrate v soybean oil, $P < .01$, safflower oil v fish oil and palm oil; $P < .05$, carbohydrate v perilla oil and fish oil; $P < .05$, safflower oil v lard oil and perilla oil.

high-carbohydrate diet 20%, 40%, 60%, and 80% increases of the glucose load resulted in only 12%, -8%, 12%, and 23% increases of blood glucose levels 30 minutes after an oral glucose challenge, which was not significant ($n = 3$).

In comparison to the high-carbohydrate diet (85 pmol/L), fasting blood insulin significantly increased by threefold with the palm oil diet (245 pmol/L, $P < .001$), but the other values were not significant (Table 3). Fasting triglyceride and cholesterol levels changed reciprocally (Table 3). Thus, in comparison to the high-carbohydrate diet (71 mg/dL), triglyceride levels decreased with lard (52 mg/dL, $P < .05$) and rapeseed oil (41 mg/dL, $P < .001$), soybean oil (42 mg/dL, $P < .01$), safflower oil (35 mg/dL, $P < .001$), perilla oil (42 mg/dL, $P < .001$), and fish oil (36 mg/dL, $P < .001$) diets, whereas total cholesterol levels increased with palm oil (97 mg/dL, $P < .001$) and lard (93 mg/dL, $P < .001$) diets compared with the high-carbohydrate diet (78 mg/dL). These findings are in good agreement with previous reports that most of the dietary vegetable oils were hypotriglyceridemic and dietary animal oils containing saturated fatty acids were hypercholesterolemic.^{23,24} It has also been reported that in comparison to a high-fat diet, a high-carbohydrate diet causes hypertriglyceridemia by elevation of very-low-density lipoprotein cholesterol levels.²⁵

Because of interactions between the final body weight and fatty acid composition, stepwise multiple regression analysis was used to determine the independent predictors

of glycemic control. The sum of glucose levels after glucose challenge (Σ glucose) was used as a marker of glycemic control. The actual intake of fatty acids from each group was calculated from the mean food intakes (Table 3) and used in this analysis. The n-3 fatty acid (18:3, 20:5, and 22:6) intake, n-6 (18:2 and 20:4)/n-3 ratio, polyunsaturated to saturated fatty acid ratio, final body weight, WAT weight, and fasting insulin, triglyceride, and cholesterol levels were also included in this regression model. Table 4 shows the partial correlation coefficients relating these variables to Σ glucose. (A higher F value indicates a higher contribution to Σ glucose.) When all variables were analyzed together, positive relations were found between Σ glucose and WAT weight, Σ glucose and final body weight, and Σ glucose and intake of linoleic acid. Negative relations were found between Σ glucose and intake of palmitic acid, and Σ glucose and n-3 fatty acids. When WAT weight was chosen as the first independent variable, the correlation with final body weight and with n-3 fatty acid intake disappeared, but the correlation with linoleic and palmitic acid still remained. Interestingly, the n-6/n-3 ratio appeared as the second largest independent variable. When both WAT and n-6/n-3 ratio were chosen as independent variables, the correlation with intake of linoleic or palmitic acid disappeared completely. Two independent variables, WAT weight and n-6/n-3 ratio, were found to explain 42% of Σ glucose variations (data not shown).

DIETARY OILS ON OBESITY AND HYPERGLYCEMIA

1643

Table 4. Stepwise Multiple Regression Analysis

Variable	(A) Not Adjusted		(B) Adjusted for WAT		(C) Adjusted for n-6/n-3 and WAT	
	r	F	r	F	r	F
16:0 (palmitic)	-.37	5.5	-.33	4.0	-.22	1.6
18:0 (stearic)	-.02	0.01	-.03	0.03	.10	0.35
18:1 (oleic)	-.08	0.20	-.14	0.62	.02	0.01
18:2 (linoleic)	.42	7.3	.32	3.8	-.12	0.43
18:3 (linolenic)	-.06	0.11	-.10	0.33	-.01	0.00
n-3	-.36	5.2	-.06	0.12	.18	1.1
n-6/n-3 ratio	.31	3.7	.40	6.5	NA	NA
P/S ratio	.28	2.9	.27	2.6	.05	0.09
Body weight	.46	9.3	-.16	0.91	-.16	0.86
WAT weight	.55	15	NA	NA	NA	NA
Triglyceride	.08	0.22	-.005	0.001	.10	0.31
Cholesterol	.14	0.64	-.02	0.01	.04	0.04
Insulin	.09	0.27	-.11	0.40	-.12	0.43

NOTE. Partial correlation coefficients and F values* are shown (A) before and (B) after adjustment for the effects of WAT weight and (C) after adjustment for the effect of WAT weight and the n-3/n-6 ratio between Σ glucose and many variables such as the major fatty acid and n-3 fatty acid intakes, n-6/n-3 and polyunsaturated to saturated fat ratios, final body weight, WAT weight, and fasting triglyceride, cholesterol, and insulin levels.

Abbreviations: P/S, polyunsaturated to saturated fat ratio; NA, not applicable.

*A larger F value indicates a larger contribution to Σ glucose.

The contribution of obesity to Σ glucose was further investigated by univariate analysis. When individual mice from all groups were plotted, Σ glucose was well correlated with body weight (Fig 2A: $r = .67$, $P < .0001$, $n = 47$). Also, Σ glucose was well correlated with wet WAT weight (data not shown: $r = .67$, $P < .0001$, $n = 47$). However, there were large variations in body weight increases among individual mice even within specific oil diet-fed groups (Fig 2A). For example, in lard oil-fed mice, the correlation between Σ glucose and body weight of individual mice was high (data not shown: $r = .85$, $P = .008$, $n = 7$). To examine the effects of various oils and individual differences on obesity-induced hyperglycemia separately, the mean body weight of each group of mice was plotted against Σ glucose. When analyzed as a group, there was no significant correlations between body weight and Σ glucose (Fig 2B: $r = .60$, $P = .12$, $n = 8$).

Since the contribution of n-3 fatty acids is minimal after adjustment of WAT weight (Table 4), n-6 intake (mostly linoleic acid) may be responsible for the favorable effects of the n-6/n-3 ratio. Univariate analysis also supported this conclusion. There was a positive correlation between Σ glucose and the actual intake amount of linoleic acid (Fig 3A: $r = .83$, $P = .02$, $n = 7$). However, there was no significant correlation between the mean final body weight and the intake amount of linoleic acid (Fig 3B: $r = .46$, $P = .30$, $n = 7$). There was no significant correlation between Σ glucose and the intake amount of n-3 fatty acid (data not shown: $r = .48$, $P = .27$, $n = 7$), but there was a significant negative correlation between the mean body weight and the intake amount of n-3 fatty acid (data not shown: $r = -.79$,

$P = .04$, $n = 7$). These data indicate that the increased intake of linoleic acid (or n-6/n-3) and obesity are independent risk factors that lead to abnormal glucose tolerance, and the favorable effects of n-3 fatty acid may be due to the decrease of body weight gain.

The intake of palmitic acid was inversely correlated with Σ glucose by multiple regression analysis. The possibility that a higher intake of palmitic acid is better for glycemic control is highly unlikely, because the intake of saturated fatty acid has been reported to induce insulin resistance in humans.²⁶ Rather, it is simply explained that because of an inverse correlation between the composition of palmitic and linoleic acids in dietary oil subtypes (Table 2), both variables may be chosen in multiple regression analysis. Also, the high n-3/n-6 ratio in the fish oil diet raises a possibility of linoleic acid deficiency.

To ascertain the basis for the loss of glycemic control caused by feeding high-fat diets, levels of GLUT4 protein in gastrocnemius (skeletal muscle) were assessed by Western blot analysis. Low-level overexpression of GLUT4 in transgenic mice is known to prevent high-fat diet-induced hyperglycemia.²⁷ In comparison to the high-carbohydrate diet, the high-fat diet subtypes resulted in a slight decrease

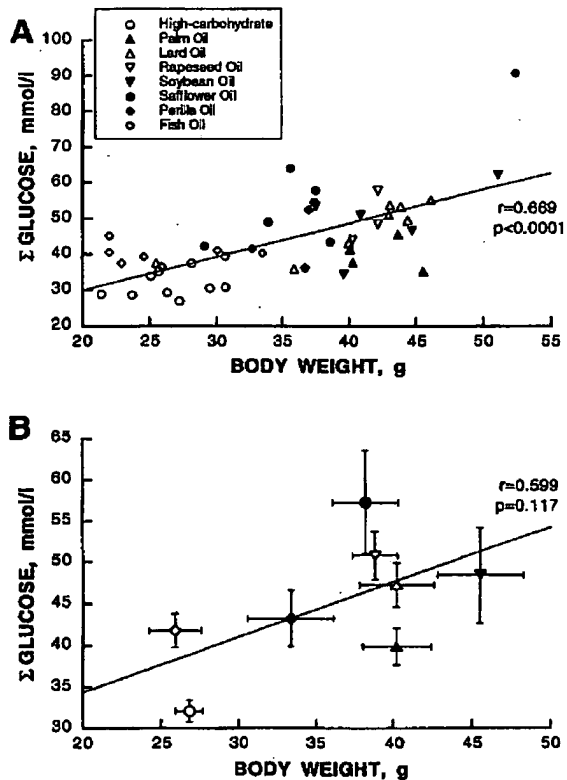


Fig 2. (A) Relationship between the body weight of individual mice and Σ glucose at 0, 30, 90, and 120 minutes after oral glucose tolerance tests; $r = .699$, $P < .0001$, $n = 47$. (B) Relationship between the mean body weight of each group and Σ glucose; $r = .599$, $P = .117$, $n = 8$.

1544

KEMOTO ET AL

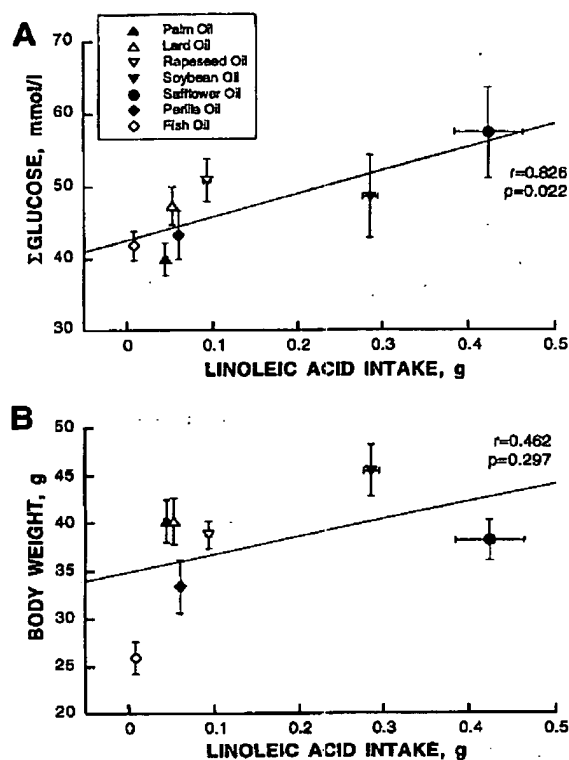


Fig 3. (A) Relationship between intake of linoleic acid and Σ glucose at 0, 30, 90, and 120 minutes after oral glucose tolerance tests. The mean linoleic acid intakes (18:2) for each high-fat group were plotted against Σ glucose; $r = .826$, $P = .022$, $n = 7$. The actual intake of oleic and linoleic acids was calculated from the amount of daily food intake shown in Table 3. (B) Relationship between linoleic acid intake and final body weight. The mean linoleic acid intakes (18:2) for each high-fat group were plotted against the mean body weight; $r = .462$, $P = .297$, $n = 7$.

of GLUT4 levels, but these were not significant by ANOVA (Fig 4).

DISCUSSION

Most of the high-fat diets in comparison to the high-carbohydrate diet were found to have adverse effects on glucose tolerance and obesity. In human studies, linoleic acid and saturated fat and oleic acid have been linked to the development of insulin resistance²⁶ and coronary artery disease.^{28,29} In this study, both obesity and intake of linoleic acid were found to be independent positive variables for aggravation of glucose tolerance.

Obesity (or WAT weight) was first chosen as an independent variable to determine the glucose tolerance. WAT weight was measured as a marker of visceral obesity. In this strain of mice, Rebuffe-Scrive et al³⁰ reported that the increase of visceral fat seemed to be a specific characteristic associated with the genetic predisposition for NIDDM. The contribution of visceral fat to abnormal glucose tolerance has been observed in mice and in humans.^{31,32}

Using a euglycemic clamp technique, Storlien et al¹² reported that replacement of only 6% of the linoleic acid from safflower oil with n-3 fatty acid from fish oil prevented the development of insulin resistance. In our study, fish and perilla oils that contained a higher amount of n-3 fatty acids showed a less diabetic glucose tolerance curve, but failed to appear as an independent variable. This is because n-3 fatty acid intake has an inverse correlation with obesity (Table 4). It has been reported that in genetically obese *ob/ob* mice, an increase in the n-3 fat content of the diet decreases weight gain despite higher intake.³³ As indicated by Pan and Storlien,³⁴ the lower weight gain associated with increased tissue levels of n-3 fatty acid might be explained by the "leaky membrane" hypothesis proposed by Else and Hulbert.³⁵ This hypothesis was further supported by the finding that the increasing amount of membrane n-3 fatty acid resulted in increased proton influx in the rat liver mitochondria, and then resulted in energy consumption.³⁶ A possible reason for the lack of change in glycemic control with fish oil supplements to humans may be the failure to reduce body weight.^{15,17} Indeed, when fish plus safflower oil-fed rats became obese, insulin resistance was manifested in adipocytes.³⁷

Oral glucose tolerance tests are affected by many factors, such as absorption rate in the intestine, insulin secretion, glucose uptake in muscle tissues, and glucose output from the liver.³⁸ Palm oil-fed mice might have had insulin resistance, but due to an increase of insulin secretion, they showed less diabetic glycemic control. This may lead to a better glycemic control but a body weight increase. It has been reported that high-coconut oil-fed mice show higher fasting plasma insulin levels.³⁰ These oils from tropical fruits have a higher amount of saturated acids: coconut oil contains mainly 45% to 50% lauric acid (12:0), and palm oil is 45% palmitic acid (16:0). On the other hand, mice fed lard and other vegetable oils did not show hyperinsulinemia, irrespective of insulin resistance. These findings are in good agreement with previous reports that rats fed lard

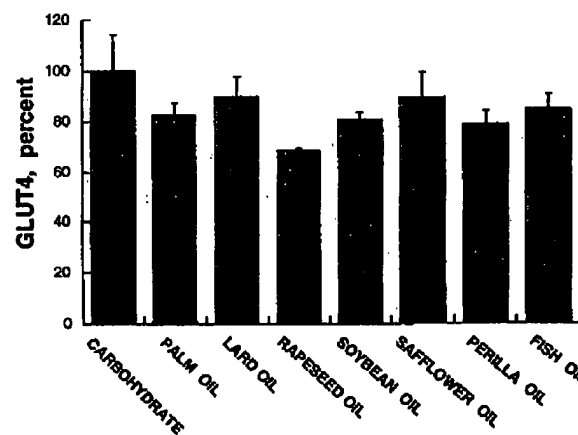


Fig 4. Effect of feeding high-fat oil subtypes on GLUT4 protein in skeletal muscles. Results are the mean \pm SE of individual mean values obtained in each of 4 mice; $F(7, 24) = 1.8$, $P = .2$.

oil³⁹ or safflower oil^{6,37} showed hypoinsulinemia. The coconut oil- and palm oil-fed mice showed a pathological state similar to that of obese hyperinsulinemic NIDDM patients observed in the United States.¹ The difference in NIDDM types between Americans and Japanese, most of whom are low insulin responders,^{40,41} may be due to a difference in intake of dietary oil types.

However, with *in vitro* studies, fatty acids *per se* are known to stimulate insulin secretion both in isolated pancreatic islets⁴² and in perfused pancreas preparations.⁴³ Also, Opara et al⁴⁴ reported that in isolated perfused murine islets, the effects of fatty acids on insulin release were dependent on the fatty acid subtype, the fatty acid-stimulated insulin secretion was strongest in 5 mmol/L 12:0 but declined with increasing chain length, and insulin secretion was enhanced as the degree of unsaturation of fatty acids increased. The discrepancy for insulin secretion between *in vitro* studies and our *in vivo* study may be explained by the fact that with the *in vitro* studies, the acute effects of lipid or free-fatty acid administration on insulin secretion were examined, whereas in our studies the chronic effects were examined. Indeed, Sako and Grill⁴⁵ reported that hyperlipidemia was associated with short-term stimulation but long-term inhibition of glucose-induced insulin secretion.

Although linoleic acid is an independent variable, linoleic acid-induced obesity also contributed to the glucose intolerance. It is clear by stepwise regression analysis that *F* values for linoleic acid decreased from 7.3 to 3.8 even after consideration of obesity. However, it has not been ruled out that other substances included in these oils but not measured in this study, such as *trans*-fatty acids,⁴⁶ have predominant effects on glucose tolerance.

Assuming that higher intakes of most dietary oils result in

abnormal glucose tolerance, how are the effects interpreted? Euglycemic clamp studies indicated that rats fed linoleic acid-rich oils showed insulin resistance in skeletal muscles and liver.⁶ The mechanisms of high-fat-induced insulin resistance are not clear at present. A decreased GLUT4 intrinsic activity, decreased translocation of GLUT4, or decreased signaling from the insulin receptor to GLUT4-containing vesicles might be involved. However, it has not been ruled out that a small decrease in GLUT4 by high-fat feeding, which was not significant in this study, might be responsible for the changes in glucose uptake observed with the dietary manipulation. Another reason is that most of the vegetable oils are not hyperinsulinemic^{6,37,39} (Table 3). Kim et al⁴⁷ proposed that decreased GLUT2 and glucokinase mRNA in pancreatic β cells are responsible for the high-fat-induced decrease of insulin secretion, although they did not describe fat types.

It was found in this study that (1) fasting blood insulin levels varied among fat subtypes: palm oil is hyperinsulinemic, but most of the other vegetable oils are not; (2) a favorable glucose response obtained by fish oil feeding may be mediated by a decrease of body weight; and (3) obesity and a higher intake of linoleic acid are independent risk factors for aggravation of glucose intolerance. However, we cannot rule out that humans might respond differently to dietary fat. Further human studies are necessary to verify this hypothesis.

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Linoleic-acid-enriched diet: long-term effects on serum lipoprotein and apolipoprotein concentrations and insulin sensitivity in noninsulin-dependent diabetic patients^{1,2}

Robert J Heine, Cees Mulder, Corrie Popp-Snijders, Jan van der Meer, and Ed A van der Veen

ABSTRACT Long-term (30 wk) effects on serum lipoproteins and insulin sensitivity of two diets, one with a low polyunsaturated to saturated fat ratio (P:S 0.3) and one with a P:S of 1.0, were compared in 14 patients with noninsulin-dependent diabetes mellitus (NIDDM) in a crossover study. Total and LDL-cholesterol levels declined by 7.6% ($p < 0.01$) and 9.8% ($p < 0.01$), respectively, during the high P:S diet. VLDL-, HDL2-, and HDL3-cholesterol; triacylglycerol; and apolipoprotein A1, A2, and B levels were not affected by the change in P:S. Despite a modest increase of insulin-mediated glucose disposal at physiologic insulinemia during the high P:S diet, no influence was seen on glycemic control, and on blood glucose, plasma insulin, and C peptide responses to mixed meals.

In conclusion, a linoleic-enriched diet in patients with NIDDM causes a less atherogenic lipoprotein profile but does not influence glycemic control and carbohydrate tolerance. *Am J Clin Nutr* 1989;49:448-56.

KEY WORDS Linoleic acid, polyunsaturated fatty acids, diet, insulin sensitivity, lipids, cholesterol, LDL-cholesterol, diabetes, apolipoproteins, lipoproteins

Introduction

Atherosclerosis is a major cause of death and morbidity in diabetic patients (1-3). Because of this predisposition, attention has been focused on lipoprotein and apolipoprotein metabolism in diabetics. Hyperlipidemia is frequently encountered in noninsulin-dependent diabetics and is considered as a major determinant of atherosclerotic complications (4-6). Besides the known hypertriacylglycerolemia, alterations in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) metabolism have attracted more attention as they may be more intrinsically related to the pathogenesis of atherosclerosis (7, 8). Also obesity, insulin resistance, and hypertension have been shown to have a predictive value for the development of coronary heart disease (1, 9, 10). These risk factors can be influenced by dietary treatment. The World Health Organization (WHO) expert committee on diabetes mellitus (11) recommends in their latest report a dietary fat restriction to approximately 30 energy percent. Moreover, they advised diabetics to substitute foods containing polyunsaturated vegetable oils for saturated fats. Consequently the carbohydrate content has to be raised to 50 energy %, enriched with natural dietary fibers. This prudent diet has also been recommended to the general population to reduce the risk of atherosclerotic events (12).

Polyunsaturated fatty acids have been claimed to enhance the sensitivity to insulin in diabetic patients. Kinsell (13) observed hypoglycemic episodes when linoleic acid was substituted for saturated fatty acids in the diets. A study in noninsulin-dependent diabetics demonstrated improvement of the glucose tolerance in women using a diet with a high ratio of polyunsaturated to saturated fatty acid (P:S) (14).

Despite these studies it is still very hard to draw firm conclusions as to the effect of polyunsaturated fatty acids on lipid levels and insulin action in general, and of linoleic acid in particular, because studies were either of short duration (13) or were comparing diets that differed in their relative proportions of carbohydrate and fat (14).

We decided therefore to study the long-term effects of a diet enriched with linoleic acid to a P:S of ~1, without further altering the composition of the diet, on serum lipoprotein and apolipoprotein levels and insulin sensitivity in noninsulin-dependent diabetic patients.

¹ From the Departments of Internal Medicine, Clinical Chemistry, and Endocrinology, Free University Hospital, Amsterdam, The Netherlands.

² Address reprint requests to RJ Heine, Department of Internal Medicine, Free University Hospital, De Boelelaan 1117, Amsterdam, The Netherlands.

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DIETARY LINOLEIC ACID IN NIDDM

449

TABLE 1

Clinical and biochemical details at time of recruitment of the patients who completed the study

Subject	Sex	Age	BMI*	Known diabetes duration	Therapy	Fasting HbA1c blood glucose†	Fasting plasma insulin	Fasting plasma C peptide	Total cholesterol	LDL cholesterol	HDL2 cholesterol	Triacylglycerol
		y	kg/m ²	y		mmol/L (%)	mU/L	nmol/L	mmol/L	mmol/L	mmol/L	mmol/L
1	M	46	29.3	8	diet	10.8 (10.4)	18	0.54	5.06	3.29	0.19	2.5
2	F	40	24.9	16	diet	13.0 (11.6)	9	0.27	8.31	6.62	0.26	1.7
3	F	55	34.0	2	diet	6.6 (6.4)	18	0.73	7.44	5.66	0.25	2.1
4	M	30	25.3	3	diet	9.6 (8.8)	11	0.30	3.55	2.18	0.23	1.2
5	F	43	24.7	5	diet	12.2 (10.5)	24	0.38	6.67	4.99	0.32	3.1
6	F	59	25.6	2	diet	8.1 (6.4)	15	0.75	5.97	3.67	0.24	5.4
7	F	61	22.8	3	glibenclamide	9.4 (9.9)	12	0.47	4.21	2.53	0.35	1.7
8	F	46	19.8	9	tolbutamide	11.5 (10.0)	15	0.28	4.33	2.98	0.40	1.6
9	M	70	28.4	8	gliclazide	7.7 (7.6)	16	0.57	5.45	4.45	0.16	1.5
10	M	69	19.5	9	glibenclamide	7.5 (10.3)	14	0.29	3.83	2.01	1.13	0.5
11	M	64	26.4	5	tolbutamide	11.5 (8.6)	10	0.43	4.16	3.03	0.23	1.0
12	M	47	24.6	8	glibenclamide	6.0 (6.0)	11	0.30	3.94	2.53	0.29	0.8
13	M	47	25.6	9	gliclazide	10.2 (6.6)	21	0.87	5.60	3.96	0.21	3.1
14	M	49	24.9	6	glibenclamide	9.5 (8.4)	10	1.12	8.14	5.96	0.35	3.6

* Body Mass Index = body wt/ht².

† Percent is given in parentheses.

Subjects and methods

Subjects

Seventeen patients, 8 women and 9 men, were recruited. Inclusion criteria for this study were age < 70 y and treatment consisting of diet only or a stable dose of sulfonylurea for at least 3 mo before the start of the study. Excluded were patients with other endocrine diseases, liver or renal disease as confirmed by biochemical findings, and those on treatment that would affect lipid or carbohydrate metabolism (eg, antihypertensives, corticosteroids, metformin, and diuretics). Patients were only admitted when it was to be expected that metabolic control did not require imminent alterations in treatment. All patients had a stable weight (< 5% different from that 3 mo before the start of the study) during at least 3 mo before the start of the study. Three patients dropped out during the first 3 mo after the start of the study, one man because of high alcohol intake that required special treatment and one woman because of breast carcinoma. In one woman metabolic control deteriorated, reflected by fasting blood glucose levels > 15 mmol/L, which required insulin treatment. Enrollment of the patients took place between April 1984 and July 1985.

All patients were diagnosed as being diabetic according to the criteria as recommended by the WHO study group on diabetes mellitus (11). The clinical characteristics of the patients who completed the study are given in Table 1. Before entering the study each patient gave informed consent and approval for the study was obtained from the Ethical Committee of the Free University Hospital was obtained.

Study protocol

The study consisted of two periods of 30 wk each in a cross-over design. During one period the P:S ratio in the diet was designed to be 0.3 and in the other period 1.0. The order of the dietary periods was randomized. At entry in the study the diets were individually planned to be isocaloric with the energy intake of the patient calculated from 1-wk dietary recalls. A com-

puter data bank of Dutch food composition was used to assess the diet compositions and to design the appropriate diet for the study. No attempt was made to adjust the habitual diet of the patient to the dietary guidelines, which were given at time of diagnosis of diabetes (ie, a diet consisting of 50–55 energy % carbohydrate, enriched with dietary fibers and 30 energy % fat with a P:S of 1). The composition of the diet and the cholesterol and fiber content were kept constant. Only the P:S of the diet was altered by substituting linoleic-acid-rich oils and fats for products rich in saturated fatty acids.

Diet compliance was controlled by 1-wk dietary recalls at 6, 12, and 24 wk during the two dietary periods. If necessary the diet prescriptions were adjusted. In addition, the fatty acid composition of the plasma cholesteryl-esters were determined at 6-wk intervals throughout the study and the lipid composition of the red blood cells at the end of the dietary periods.

The patients were seen at 6-wk intervals in the outpatient clinic after an overnight fast from 2200 the evening before, for the determination of body weight and blood pressure. Blood pressure was measured after a 15-min rest in a sitting position by one observer using the same mercury sphygmomanometer with a standard cuff. Diastolic blood pressure was recorded at Korotkoff phase V. The mean of three measurements was recorded.

Blood was withdrawn from an antecubital vein for the assessments of blood glucose, hemoglobin A_{1c} (HbA_{1c}), serum lipids (total cholesterol, triacylglycerol, very-low density lipoprotein [VLDL] cholesterol, LDL cholesterol, HDL2 and HDL3 cholesterol), and apolipoproteins A₁, A₂, and B. After taking the blood samples, 100 IU heparin/kg body wt (Organon Teknika BV, Bostel, Holland) was administered as an intravenous bolus. Twenty minutes later venous samples (EDTA as anticoagulant) were withdrawn for the assessments of post heparin lipolytic activity (PHLA), lipoprotein lipase (LPL), and hepatic lipase activity. Blood for measurement of ¹²⁵I-labeled insulin binding to red blood cells, according to Gambhir et al, was taken at 18 and 30 wk of each dietary period (15).

450

HEINE ET AL

TABLE 2

Nutrient composition of the reference (low P:S) diet and the linoleic-acid enriched (high P:S) diet (mean 24-h values of 1-wk dietary recalls)

	Low P:S diet				High P:S diet			
	Week 6	Week 12	Week 24	Mean	Week 6	Week 12	Week 24	Mean
Energy intake (kcal/d)	1666 ± 86	1746 ± 117	1826 ± 120	1746 ± 98	1641 ± 105	1752 ± 112	1738 ± 113	1710 ± 104
Protein								
animal (energy %)	10.7 ± 0.8	12.1 ± 0.8	11.6 ± 0.9	11.5 ± 0.7	12.8 ± 1.0	12.7 ± 0.9	12.1 ± 1.0	12.5 ± 0.9
vegetable (energy %)	4.8 ± 0.4	4.7 ± 0.3	4.7 ± 0.3	4.7 ± 0.3	5.0 ± 0.4	4.9 ± 0.4	4.8 ± 0.4	4.9 ± 0.2
Fat (energy %)	38.6 ± 2.1	38.9 ± 2.0	39.7 ± 2.3	39.1 ± 2.1	37.0 ± 1.7	38.3 ± 1.8	39.3 ± 1.5	38.2 ± 1.5
linoleic acid (energy %)	3.9 ± 0.4	4.1 ± 0.5	4.6 ± 0.4	4.2 ± 0.4	10.0 ± 0.8	10.9 ± 0.8	11.7 ± 0.8	10.9 ± 0.6†
P:S	0.31 ± 0.03	0.32 ± 0.03	0.35 ± 0.03	0.33 ± 0.02	0.83 ± 0.07	0.93 ± 0.08	0.97 ± 0.08	0.91 ± 0.06†
Cholesterol (mg/d)	261 ± 25	266 ± 28	290 ± 33	272 ± 25	217 ± 20	225 ± 21	223 ± 14	222 ± 13‡
Dietary fiber (g/d)	20 ± 2	21 ± 2	22 ± 2	21 ± 2	22 ± 2	21 ± 2	20 ± 2	21 ± 2
Carbohydrate (energy %)	40.2 ± 1.9	39.4 ± 1.9	38.8 ± 2.1	39.5 ± 1.9	39.9 ± 1.6	40.4 ± 1.6	39.4 ± 1.2	39.9 ± 1.3
Alcohol (energy %)	5.9 ± 1.4	5.1 ± 1.4	5.2 ± 1.4	5.4 ± 1.3	5.2 ± 1.3	4.0 ± 1.3	4.6 ± 1.2	4.6 ± 1.2

* $\bar{x} \pm 1$ SEM.† $p < 0.001$ as compared with mean value during low P:S diet.‡ $p < 0.05$.

Meal tolerance tests were performed in the fasting state at 0800 during the final 2 wk of the dietary periods. A standardized liquid mixed meal containing 56 energy % carbohydrate and 25 energy % protein (Clinifeed-protein rich®, Roussel, Hoevelaken, Holland) was taken in 5 min. Blood glucose and plasma insulin and C peptide were measured twice basally and at 15-min intervals for 3 h thereafter.

In vivo insulin sensitivity was assessed by constructing insulin dose-response curves in the fasting state, separated by at least 1 wk from the meal tolerance test, at the end of the dietary periods, by use of sequential glucose and insulin infusions at prefixed rates (16). Insulin was infused sequentially at 50, 150, and 500 mU·kg⁻¹·h⁻¹ (Humulin R, Lilly, Indianapolis, IN) for 150, 120, and 120 min, respectively. Each insulin infusion was primed by an intravenous bolus injection of insulin of 0.1 × kg body wt × desired elevation of plasma insulin levels in mU. Glucose infusions at 6, 8, and 10 mg·kg⁻¹·min⁻¹ were initiated 10 min later than the corresponding insulin infusions. Blood samples for blood glucose levels were taken each 5 min and for plasma insulin at 10-min intervals during the final 30 min of each glucose-insulin infusion. From these values the steady state plasma insulin levels were calculated. The metabolic clearance rate of glucose was obtained by dividing the glucose infusion rate by the mean blood glucose level during the final 30 min of each infusion.

Laboratory methods

Blood glucose was measured by a glucose oxidase method (Yellow Springs Instrument Co, Yellow Springs, OH). Plasma insulin was assessed by radioimmunoassay with human insulin as standard (Sorin Biomedica, Sallugia, Italy; sensitivity 2.5 mU/L; intraassay coefficient of variation 8.2%). Plasma C peptide was measured by radioimmunoassay with ethanol precipitation with human C peptide as standard (Novo Industri, Bagsvaerd, Denmark; sensitivity 0.06 nmol/L, intraassay coefficient of variation 8.1%) (17). Glycosylated hemoglobin was assayed by a microcolumn method (18). For the determination of triacylglycerol levels the glycerol phosphate oxidase-para amino phenozone (GPO-PAP) (human) was applied. Total cholesterol in serum and in the lipoprotein fractions was measured enzymatically using the Monotest kit (Boehringer Mann-

heim, GmbH, FRG). An IEC-B60 ultracentrifuge (Damon/IEC, Needham Heights, MA) was used for density gradient ultracentrifugation: VLDL was isolated at $d < 1019$ g/mL LDL between $d = 1019$ and $d = 1063$ g/mL, HDL2 between $d = 1063$ and $d = 1.125$ g/mL, and HDL3 between $d = 1.125$ and $d = 1.210$ g/mL (19). The PHLA was measured according to a modified method of Lewis (20).

Post heparin plasma was incubated with Intralipid® (Kabi-Vitrum, Stockholm, Sweden) and bovine serum albumin with a negligible free fatty acid content (Boseral, Organon, Oss, Holland) in a tris-HCl buffer (0.2 mol tris/L, 0.15 mol NaCl/L, pH = 8.5). For the determination of released fatty acids the enzymatic nonesterified fatty acid-C (NEFA-C) test (Wako, Osaka, Japan) was used (21). To determine hepatic triacylglycerol lipase (h-TGL), the same assay was used with selective inhibition of lipoprotein lipase by protamine sulfate (22). Subtraction of h-TGL from PHLA gives information about the LPL activity.

Apolipoprotein A1 and A2 were measured using a modification of the immunoturbidimetric assay, which was based on an immunonephelometric technique (23). Apolipoprotein B was measured by radial immunodiffusion.

The fatty acid composition of the plasma cholesteryl esters was analyzed by gas-liquid chromatography (24). Erythrocyte membrane lipid analyses were performed as described previously (25). The fatty acid composition of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) were analyzed separately. The phospholipid unsaturation was calculated from the total fatty acid composition of PC and PE and was expressed as double bond index (DBI: mean of double bonds per molecule of fatty acid).

Statistical analysis

This study was designed as a crossover clinical trial with two study periods of 30 wk each. From the multiple measurements of the variables during the two study periods, average values of the three final measurements obtained between weeks 18 and 30 were calculated. For the analysis, the data from the two sequence groups were pooled. For the statistical evaluation of the treatment responses analysis of variance according to Hills and Armitage was performed (26). This evaluation included the

DIETARY LINOLEIC ACID IN NIDDM

451

TABLE 3

Fatty acid composition of cholesteryl esters during the study periods with a low P:S and high P:S diet (mean values of three assessments at weeks 18, 24, and 30)*

	Fatty acid	mol %	
		Low P:S diet	High P:S diet
Palmitic	(C _{16:0})	13.0 ± 0.4	12.4 ± 0.2
Palmitoleic	(C _{16:1})	3.9 ± 0.5	3.3 ± 0.5†
Stearic	(C _{18:0})	1.1 ± 0.1	1.1 ± 0.1
Oleic	(C _{18:1})	18.4 ± 0.6	15.7 ± 0.9‡
Linoleic	(C _{18:2})	55.8 ± 1.5	59.8 ± 2.0‡
Linolenic	(C _{18:3})	1.1 ± 0.1	1.0 ± 0.2
Homogammalinolenic	(C _{20:3})	0.6 ± 0.1	0.6 ± 0.1
Arachidonic	(C _{20:4})	5.1 ± 0.3	5.0 ± 0.4
Eicosapentaenoic	(C _{20:5})	0.7 ± 0.1	0.7 ± 0.1
Docosahexaenoic	(C _{22:6})	0.3 ± 0.1	0.4 ± 0.1
	DBI§	1.65 ± 0.02	1.70 ± 0.01†

* $\bar{x} \pm 1$ SEM.† $p < 0.01$.‡ $p < 0.001$ as compared to mean value during low P:S diet.

§ DBI (Double Bond Index) = mean of double bonds per molecule fatty acid.

analysis of the sequence effect on the results. All results are presented as means \pm SEM.

Results

Diet compliance was determined with two independent methods: 1-wk dietary recalls at weeks 6, 12, and 24 and assessment of the fatty acid composition of the cholesteryl esters at 6-wk intervals (Tables 2 and 3). The cholesterol intake was lower during the high P:S as compared with the low P:S diet ($p < 0.05$). The composition of the diet remained otherwise unchanged (Table 2). These dietary recall data were confirmed by the fatty acid composition of the cholesteryl esters, which demonstrated a significant increase of the linoleic acid (C_{18:2}) content, mainly at the expense of palmitoleic acid (C_{16:1}) and oleic acid (C_{18:1}). These changes resulted in a significant increase of the DBI (Table 3).

The relevant fatty acid content in erythrocyte PC and PE are given in Table 4. The high P:S diet caused a significantly higher stearic acid (C_{18:0}) and linoleic acid (C_{18:2}) content in PC at the expense of C_{16:0} and oleic acid. In erythrocyte PE the high P:S diet, as compared with the low P:S diet, resulted in a significantly higher linoleic acid and stearic acid content and a lower oleic acid content. In neither PC nor PE was a change seen in the total ω -3 fatty acid content or in the DBI.

Mean body weights were not significantly different for either dietary period: 77.9 \pm 4.0 vs 78.0 \pm 4.1 kg for the low vs high P:S diets, respectively. Mean systolic and diastolic blood pressures were not affected by the change in dietary linoleic acid intake: 137.6 \pm 4.4 vs 138.1 \pm 3.8 mm Hg and 84.8 \pm 2.0 vs 85.0 \pm 2.0 mm Hg for the low and high P:S diets, respectively.

Serum lipoprotein, apolipoprotein, and triacylglycerol levels are given in Figure 1 and Table 5. Total cholesterol and LDL-cholesterol levels were significantly higher during the low P:S as compared with the high P:S diet (Table 5). The differences in total cholesterol level were only obvious from 12 wk onward (Fig 1). Apolipoprotein B levels tended to be lower during the high P:S diet than during the low P:S diet but the mean levels during the final 12 wk were not significantly different for either dietary period. Serum VLDL, HDL2 and HDL3 cholesterol, apolipoprotein A1 and A2, and triacylglycerol levels were not significantly different for the low and high P:S diets.

The PHLA and the activities of hepatic and LPL were not influenced by the change in P:S of the diet: 19.9 \pm 1.2 vs 20.0 \pm 0.6, 6.4 \pm 0.7 vs 6.7 \pm 0.6, and 13.5 \pm 0.8 vs 13.3 \pm 0.9 mmol·L⁻¹·h⁻¹ for the low and high P:S diets, respectively.

Fasting blood glucose levels and glycosylated hemoglobin percentages were not significantly different for the low and high P:S diets: 10.8 \pm 0.7 vs 10.5 \pm 0.7 mmol/L and 9.2 \pm 0.7 vs 9.2 \pm 0.7%, respectively.

Incremental blood glucose, plasma C peptide, and insulin responses to the standardized liquid mixed meal during the final week of the study periods were not significantly different for either diet: 577 \pm 91 vs 597 \pm 79 mmol·L⁻¹·min⁻¹, 5199 \pm 1260 vs 4531 \pm 909 mU·L⁻¹·min⁻¹, and 169 \pm 31 vs 175 \pm 33 nmol·L⁻¹·min⁻¹ for the low and high P:S diets, respectively.

In vitro binding of ¹²⁵I-labeled insulin at tracer concentrations to red blood cells was significantly greater during the dietary period with a high linoleic acid intake as compared with the saturated fat intake: 7.4 \pm 1.3 vs 6.2 \pm 0.2% ($p = 0.003$).

In vivo insulin sensitivity was assessed by constructing insulin dose-response curves at three different infusion rates. The fasting plasma insulin levels at the end of each dietary period were identical during the low and high P:S diet: 9 \pm 1 vs 9 \pm 1 mU/L. The plasma insulin levels achieved during the dietary periods with a low and high P:S ratio were not significantly different for the three insulin infusion rates (Table 6), permitting comparison of the metabolic clearance rate of glucose. From the blood glucose levels achieved during the final 30 min of each glucose-insulin infusion, the metabolic clearance rate (MCR) of glucose was calculated. The MCR of glucose was significantly higher at the insulin infusion rate of 50 mU·kg⁻¹·h⁻¹ during the dietary period with a high P:S as compared with a low P:S diet (Table 6). At the two higher insulin infusion rates the MCRs of glucose were not significantly different for either dietary period.

The serum lipids and insulin sensitivity variables responded not significantly different in subgroups of patients that were formed according to age, body mass index (BMI), sex, and fasting blood glucose level.

Discussion

The increased risk of ischemic heart disease in diabetics has been attributed to the diabetic state per se, includ-

TABLE 4
Relevant fatty acid content in erythrocyte phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the final week of the study periods with a low and high P:S diet*

		PC		PE	
Fatty acid		Low P:S diet	High P:S diet	Low P:S diet	High P:S diet
		<i>mol %</i>			
	(C _{16:0})	38.7 ± 0.4	37.9 ± 0.6†	18.7 ± 0.4	18.3 ± 0.4
Stearic	(C _{18:0})	10.8 ± 0.3	11.6 ± 0.4†	7.6 ± 0.2	8.0 ± 0.2†
Oleic	(C _{18:1})	15.5 ± 0.6	14.1 ± 0.8‡	16.6 ± 0.4	15.7 ± 0.4‡
Linoleic	(C _{18:2})	23.4 ± 1.0	24.7 ± 1.4†	7.0 ± 0.4	7.7 ± 0.6†
Arachidonic	(C _{20:4})	4.8 ± 0.3	4.8 ± 0.3	18.8 ± 0.4	18.7 ± 0.4
	ω-3§	2.2 ± 0.1	2.4 ± 0.2	9.9 ± 0.6	9.3 ± 0.7
	DBI	1.01 ± 0.01	1.04 ± 0.01	1.87 ± 0.02	1.86 ± 0.02

* $\bar{x} \pm 1$ SEM.

† $p < 0.05$.

‡ $p < 0.01$.

§ ω-3 = omega 3 fatty acids, ie, sum of 20:5, 22:5, and 22:6 fatty acids.

|| DBI (Double Bond Index) = mean of double bonds per molecule fatty acid.

ing hyperglycemia and hyperinsulinemia and to abnormalities in lipid metabolism (1-3, 9, 10, 27-29). The relationship between the development of atherosclerotic complications and the existence of hyperlipidemia is complex although the latter is suspected to contribute to the high prevalence of macrovascular complications in diabetes (6, 7, 9, 30, 31).

From the dietary record data and the plasma cholesterol ester analyses and the stable weight throughout the study periods it was concluded that the compliance of the patients to the prescribed diets was excellent, which probably was due to the simplicity of the dietary alteration, ie, substitution of linoleic-acid-enriched oils and margarines for saturated fatty acids. The major advantage of this study design is that the observed treatment effects can be attributed to changes in the linoleic-acid content of the diet only.

The cholesterol intake during the dietary period with a low P:S ratio was higher than during the use of the linoleic-acid-enriched diet, probably because of the use of butter rather than margarines and vegetable oils. However, it is very unlikely that a difference in cholesterol consumption of 50 mg/d will affect the lipoprotein levels (32, 33).

The differences in P:S of the diet were also reflected by changes in proportional fatty acid contents of PC and PE in the erythrocyte membrane. Linoleic-acid enrichment of the diet resulted in an increase of linoleic acid in both PC and PE phospholipid classes, mostly at the expense of oleic acid. The lower intake of saturated fatty acids during the high P:S diet resulted in a lower C_{16:0} content, which seemed to be compensated for by a proportional increase of stearic acid. The sums of the C_{16:0} and stearic and of the oleic and linoleic fatty acid contents in PC and PE remained remarkably similar for both dietary periods, supporting the hypothesis that cells regulate to some

extent their membrane lipid composition in an attempt to control membrane fluidity (34).

Hypertension is a known risk factor for cardiovascular disease in nondiabetic and diabetic patients (1, 2, 9). Risk of cardiovascular disease increases with increasing blood pressure. Therefore, it seems that already small reductions in blood pressure can be favorable. Another study (35) demonstrated that a diet low in fat (~23% of energy intake) with a P:S ratio of ~1 and enriched with vegetables has a modest lowering effect on the blood pressure during a 6 wk follow-up study as compared with a conventional diet (dietary fat 40 energy %, P:S 0.27) in 55 subjects. It was suggested that the increase in the P:S was the major dietary change responsible for the lowering of the blood pressure. In our long-term study no change in blood pressure was observed during the dietary period with a high P:S, suggesting that linoleic-acid enrichment of the diet has no blood pressure lowering effect. Therefore, it seems more likely that other components that were altered in the study by Puska et al (35), eg, dietary fibers (36) and total fat content (37), must be held responsible for the observed effect.

In our study no changes were observed in glycemic control, as reflected by fasting blood glucose levels and proportions of glycosylated hemoglobin, during either dietary period. Previous studies have suggested that substitution of sunflower oil for saturated fatty acids causes a decrease of insulin requirements in diabetic patients (13, 38). Houtsmuller (38) found modest improvements in blood glucose profiles in seven obese noninsulin-dependent diabetics in metabolic ward conditions during 10 d of feeding experiments comparing 50 energy % sunflower oil with 50 energy % butter. These short-term experiments comparing extreme P:S are obviously not comparable with our long-term study in which acceptable and practically maintainable diets in normal daily

DIETARY LINOLEIC ACID IN NIDDM

453

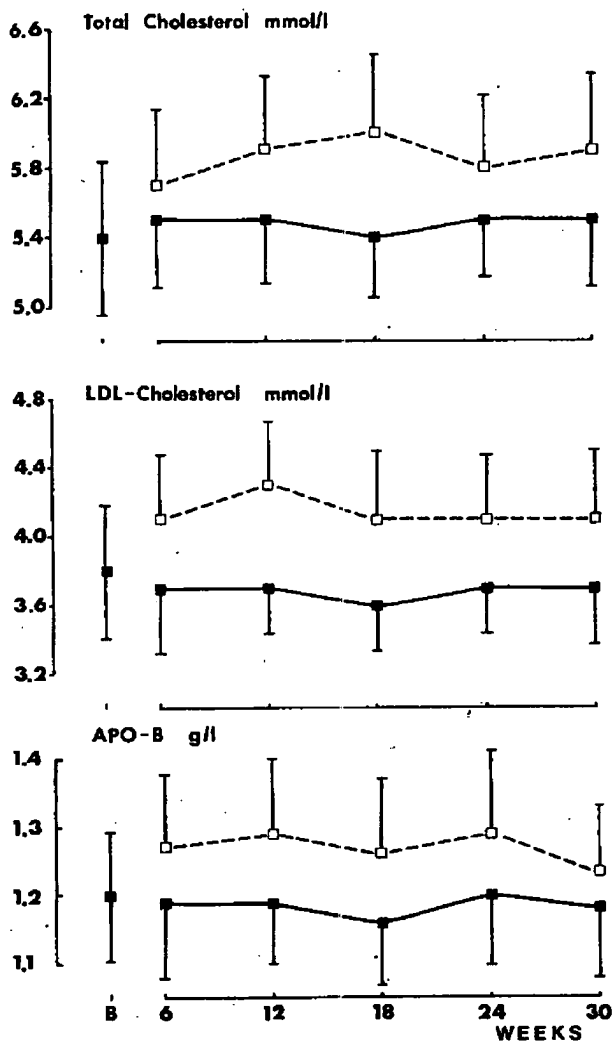


FIG 1. Total and LDL-cholesterol and apolipoprotein B (apo-B) serum levels at base line (B) and during the use of the low (□...□) and high P:S (■—■) diets for 30 wk ($\bar{x} \pm$ SEM).

life situations were investigated. From our study we may conclude that linoleic-acid enrichment to an amount of ~10 energy % without altering the other components of the diet has no effect on glycemic control in moderately well controlled noninsulin-dependent diabetic patients. Our data are in agreement with a recent study comparing a low carbohydrate (LC) diet (40 energy % fat with a P:S of 0.3) with a modified fat (MF) diet (30 energy % fat with P:S of 0.9) in a group of 149 noninsulin-dependent diabetic patients. During a 5-y follow-up no differences in glycemic control could be demonstrated between the LC and MF dietary groups (39).

One of the major pathophysiologic factors responsible for the diabetic state in noninsulin-dependent diabetes is insulin resistance (40). The impaired insulin action has

been attributed to defects at the binding site (insulin receptor) and to impeded intracellular glucose metabolism (postbinding defect). In this study we assessed the specific binding of 125 I-labeled insulin to red blood cells, as a measure of insulin receptor binding capacity (15). During the high P:S diet a significantly higher insulin binding was found as compared with the low P:S diet, which may be explained by alterations in the phospholipid milieu in which the insulin receptor is embedded. This finding is in agreement with the results of Ginsberg et al (41) who demonstrated an increase of the total number of insulin receptors on Friend erythroleukemic cells in media causing an enhancement of unsaturated fatty acids in cell membranes. However the insulin binding data do not necessarily give information on the in vivo insulin sensitivity. The fact that no direct correlation has been demonstrated between insulin-binding properties of blood cells and adipocytes in noninsulin-dependent diabetes has casted considerable doubt on its use as a measure of the insulin binding characteristics of insulin-sensitive tissues, which are relevant to carbohydrate metabolism in vivo (42).

In vivo insulin sensitivity was measured by constructing insulin dose-response curves. Insulin action was expressed as the MRC of glucose during the glucose-insulin infusions at prefixed rates. This method has been demonstrated to give comparable results with the glucose clamp technique in subjects with normal and abnormal glucose tolerance. In a previous study (16) we found in normal volunteers an increase of the mean MCR of glucose from 10.8 ± 0.6 to 20.0 ± 1.2 mL \cdot kg $^{-1}$ \cdot min $^{-1}$ when enhancing the insulin infusion rate from 50 to 500 mU \cdot kg $^{-1}$ \cdot h $^{-1}$ whereas the mean insulin dose-response curve of the patient in the present study was similar to the mean curve of a group of noninsulin-dependent diabetics which reflected impeded insulin action. The high P:S diet caused a small but significant improvement of the insulin action at insulin levels of ~50 mU/L without affecting the MCR of glucose at the higher insulin levels.

TABLE 5

Serum concentrations of lipoproteins and apolipoproteins during the study periods with a low and high P:S diet (mean values of three assessments at weeks 18, 24, and 30)*

	Low P:S diet	High P:S diet	Percent
Total cholesterol (mmol/L)	5.92 ± 0.45	$5.47 \pm 0.35^\dagger$	7.6
VLDL cholesterol (mmol/L)	0.74 ± 0.15	0.68 ± 0.14	
LDL cholesterol (mmol/L)	4.08 ± 0.38	$3.68 \pm 0.28^\dagger$	9.8
HDL2 cholesterol (mmol/L)	0.37 ± 0.04	0.38 ± 0.05	
HDL3 cholesterol (mmol/L)	0.73 ± 0.04	0.74 ± 0.04	
Triacylglycerol (mmol/L)	2.42 ± 0.41	2.22 ± 0.34	
Apolipoprotein A1 (g/L)	1.06 ± 0.04	1.10 ± 0.03	
Apolipoprotein A2 (g/L)	0.35 ± 0.02	0.36 ± 0.02	
Apolipoprotein B (g/L)	1.26 ± 0.11	1.18 ± 0.09	

* $\bar{x} \pm 1$ SEM.

$^\dagger p < 0.01$ as compared with mean value during low P:S diet.

TABLE 6

Plasma insulin levels and metabolic clearance rates (MCR) of glucose during the sequential glucose-insulin infusions in the final week of the study periods with a low and high P:S diet*

Infusion rate		Plasma insulin		MCR of glucose	
Glucose	Insulin	Low P:S diet	High P:S diet	Low P:S diet	High P:S diet
$\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	$\text{mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$	mU/L	mU/L	$\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	$\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$
6	50	50 ± 6	50 ± 4	3.7 ± 0.6	4.9 ± 0.9
8	150	168 ± 19	161 ± 16	9.7 ± 1.4	10.2 ± 1.4
10	500	1004 ± 114	971 ± 95	15.4 ± 2.3	15.5 ± 1.8

* $\bar{x} \pm 1 \text{ SEM}$.

† $p < 0.05$ as compared with mean value during low P:S diet.

It seems from the in vitro and in vivo data that linoleic-acid enrichment of the cell membranes may affect the insulin receptor binding capacity, possibly by influencing the fatty acid composition of the cell membranes (41). However the major impediment in insulin action was not alleviated and therefore it was not to be expected that the small enhancement of insulin action at the low insulin levels was translated into an improved glycemic control.

The meal tolerance test demonstrated very similar incremental glucose, insulin, and C peptide responses to a liquid mixed meal during the final week of each dietary period, confirming the lack of a major change in insulin sensitivity and demonstrating an unaltered β cell function and hepatic insulin extraction. Therefore, it seems very unlikely that previously reported improvement of glucose tolerance in noninsulin-dependent diabetics by use of a modified fat diet is due to the linoleic acid enrichment only (14).

The serum total and LDL-cholesterol levels were significantly lower during the high P:S as compared with the low P:S diet. This difference seems to be due mainly to a rise of the serum total and LDL-cholesterol levels during the low P:S diet (Fig 1). Other studies in nondiabetic subjects, which compared diets with similar P:S and fat contents as in our study, demonstrated greater differences in total and LDL-cholesterol levels than those we found (43, 44).

Furthermore, a significant decline of apolipoprotein B levels is usually observed in nondiabetic subjects who eat diets with a high P:S ratio (43–46). Therefore, it seems that noninsulin-dependent diabetics respond differently and possibly less effectively to changes in linoleic acid content of the diet than nondiabetic subjects. Our patient population demonstrated a wide range in age, BMI, and fasting blood glucose levels. However, their serum lipid levels responded to the change in the P:S of the diet in a fairly uniform way. Also, the female and male patients did not respond significantly different to the dietary alteration. The Lipid Research Clinical Trials (47) have demonstrated that each percent reduction of the LDL-cholesterol level was associated with a 2% decrease of the incidence of coronary heart disease. Because it is

suspected that also in diabetes disordered lipoprotein metabolism may contribute to the high prevalence of macrovascular complications, it is of great interest that linoleic-acid enrichment of the diet resulted in a mean decrease of LDL cholesterol levels by 9.8% without affecting HDL2-cholesterol levels. The catabolism of the LDL particle is mediated mainly by LDL-receptor mediated pathways (48). Because one of the explanations for the cholesterol lowering effect of a high P:S diet is an enhanced LDL clearance, it is tentative to speculate that an increase of the linoleic acid content of the cell membranes may stimulate, as suggested for the insulin binding site, the activity of the LDL receptors (49). Serum levels of other major lipoproteins, triacylglycerol, and apolipoproteins A1 and A2 were not affected by the change in P:S of the diet. This finding is in agreement with previous observations (12, 44, 46) in nondiabetic subjects. Other investigators have found a triacylglycerol lowering effect and decreased HDL-cholesterol levels in healthy and hyperlipidemic subjects after high P:S diets (50, 51). These discrepancies in results may be explained by differences in composition and the P:S of the diets and by the heterogeneity of lipid disorders in the study populations. Also, the duration of the investigations might be a very important determinant for the outcome of study results. The diet-induced change in total cholesterol levels was only obvious 12 wk after initiating the change in the P:S. This raises some doubt on the interpretability of shorter term dietary studies.

The PHLA was not affected by the linoleic-acid enrichment of the diet nor were the LPL and hepatic lipase activity different for either study period. Low HDL2 concentrations in diabetes may be caused by subnormal LPL and/or high hepatic lipase activity. HDL is derived from excess surface constituents, which are released after LPL-induced hydrolysis of triacylglycerol rich particles and its further metabolism is mediated by hepatic lipase (8). LPL activity is regulated by insulin. Insulin treatment in noninsulin-dependent diabetics causes enhanced LPL activity, reflected by an increase in HDL cholesterol and a decline in triacylglycerol levels (52). The absence of an effect of linoleic acid on LPL and hepatic lipase activity

DIETARY LINOLEIC ACID IN NIDDM

455

is in accordance with the unaltered serum levels of triacylglycerol and HDL2 cholesterol in our study.

Taken together, linoleic-acid enrichment of the diet resulted in lower total and LDL-cholesterol levels without a change in the HDL2-cholesterol concentrations. Glycemic control was not affected by the high P:S diet. These effects of linoleic acid on the lipid profile are considered to be favorable, ie, antiatherogenic, and may therefore reduce the high incidence of macroangiopathic events in diabetic patients.

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*Hypothesis Paper*

IS INSULIN RESISTANCE INFLUENCED BY DIETARY LINOLEIC ACID AND TRANS FATTY ACIDS?

ARTEMIS P. SIMOPOULOS

The Center for Genetics, Nutrition and Health, Washington, DC, USA

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Abstract—The incidence of obesity, noninsulin-dependent diabetes mellitus (NIDDM), hypertension, and coronary artery disease has increased in the developed world. At the same time, major changes in the type and amount of fatty acid intake have occurred over the past 40–50 years, reflected in increases in saturated fat (from both animal sources and hydrogenated vegetable sources), trans fatty acids, vegetable oils rich in linoleic acid, and an overall decrease in long chain polyunsaturated fatty acids (arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid—C20–C22). Recent findings that C20–C22 in muscle membrane phospholipids are inversely related to insulin resistance, whereas linoleic acid is positively related to insulin resistance, suggest that diet may influence the development of insulin resistance in obesity, insulin-dependent diabetes mellitus (IDDM), hypertension, and coronary artery disease (including asymptomatic atherosclerosis and microvascular angina). These conditions are known to have genetic determinants and have a common abnormality in smooth muscle response and insulin resistance. It is proposed that the current diet influences the expression of insulin resistance in those who are genetically predisposed. Therefore, clinical investigations are needed to evaluate if lowering or preventing insulin resistance through diet by increasing arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid, while lowering linoleic acid and decreasing trans fatty acids from the diet, will modify or prevent the development of these diseases.

Keywords—Obesity, Syndrome X, Insulin resistance, Linoleic acid, Arachidonic acid, Eicosapentaenoic acid, Docosahexaenoic acid, Trans fatty acids, Noninsulin-dependent diabetes mellitus, Coronary artery disease, Hypertension, Free radicals

INTRODUCTION

Insulin resistance is a metabolic state in which insulin in physiological concentrations fails to produce a normal biologic response. Obesity is a classic state of insulin resistance. In 1988, Reaven stated that resistance to insulin-stimulated glucose uptake is present in the majority of patients with impaired glucose tolerance or noninsulin-dependent diabetes mellitus (NIDDM) and in about 25% of nonobese healthy individuals with normal glucose tolerance.¹ Reaven described "Syndrome X" as consisting of resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinemia, increased very-low-density lipoprotein triglyceride, and decreased high-density lipoprotein cholesterol and hypertension, and considered insulin resistance to be of primary importance in coronary artery disease, hypertension, and NIDDM.¹ Since then, an explosion of publications has described the relation-

ships among insulin resistance, hyperinsulinemia, hypertension, and coronary artery disease.^{2,3}

By means of the euglycemic clamp technique, insulin resistance has been found in patients with NIDDM; in obese individuals; in nonobese hypertensives; in patients with asymptomatic atherosclerosis, but having normal weight, normal glucose, and normal lipid levels⁴; and in patients with microvascular angina and normal coronary arteries.⁵

Foster pointed out that in "Syndrome X," as hypothesized by Reaven, insulin resistance and some of the components of "Syndrome X" are found in individuals who are otherwise healthy.³ In patients with NIDDM, obesity, polycystic ovary syndrome, lupus erythematosus, and other autoimmune diseases characterized by antibodies to the insulin receptor, rare disorders such as leprechaunism, partial or complete lipodystrophy, and other endocrine diseases, insulin resistance is considered secondary, and reverts to normal when the primary illness is treated or controlled. In the nonobese hypertensive patient, however, drug treatment lowers blood pressure but has no effect on

Address correspondence to: Artemis P. Simopoulos, The Center for Genetics, Nutrition and Health, 2001 S Street, N.W., Suite 530, Washington, DC 20009, USA.

the insulin resistance or hyperinsulinemia or their other metabolic effects. There is no drug available that improves insulin resistance. There is a need to further understand the pathogenesis of insulin resistance before treatment can be intelligently planned.

INSULIN RESISTANCE PRECEDES THE METABOLIC ABNORMALITIES IN "SYNDROME X"

Insulin resistance has been shown to occur in the preobese state in normotensive offspring of hypertensive parents and appears to precede both the development of overt hypertension and gain or redistribution of body fat.⁶ In a study over an 8–10-year follow up, Haffner et al. showed that high fasting insulin levels, or hyperinsulinemia, preceded the development of the metabolic abnormalities that constitute "Syndrome X".⁷ Most important, hyperinsulinemia was not related to increased low-density lipoprotein (LDL) or total cholesterol, but in multivariate analyses, after adjustment for obesity and body fat distribution, fasting insulin continued to be significantly related to the incidence of decreased high-density lipoprotein (HDL) cholesterol and increased triglyceride concentrations and to the incidence of NIDDM.⁷ These studies indicate that in some patients insulin resistance precedes obesity and the metabolic abnormalities of "Syndrome X" and provide further support for the primary role of insulin resistance. Furthermore, parental history of diabetes is a risk factor for hypertension as well as diabetes.^{8,9}

Because insulin resistance accounts for about 25% of nonobese hypertensives and for about 20% of patients with angina-like syndrome, it has been suggested that persons be screened for insulin resistance, particularly if there is family history for hypertension, NIDDM, coronary artery disease, and obesity. It should be pointed out that insulin's role in atherosclerosis was noted in the 1960s,^{10–13} and a number of metabolic studies have confirmed the importance of insulin action in "Syndrome X" and obesity.¹⁴ The hypothesis is that "Syndrome X" and obesity, whether genetically determined or acquired, might act through insulin resistance to cause the metabolic abnormalities.¹⁴ It has been recommended that "Syndrome X" be renamed "Insulin Resistance Syndrome."⁵

INSULIN RESISTANCE: DIABETES

Caro refers to Himsworth in 1936 as stating that human disease could be associated with insulin resistance.¹⁴ The cellular mechanism of insulin action is still not known. The principal site of insulin-mediated glucose disposal is the skeletal muscle. The transport

Table 1. Environmental Factors Influencing Insulin Action, Increasing Insulin Resistance, or Decreasing Sensitivity to Insulin and Secondary Hyperinsulinemia

1. Insulin action declines with increases in body weight (although in some patients, insulin resistance precedes the obese state).
2. Physical activity decreases insulin resistance.
3. Weight loss decreases insulin resistance.
4. Alcohol increases insulin resistance.
5. Saturated fat intake increases insulin resistance.
6. Linoleic acid (18:2w6) in muscle phospholipid is positively correlated to hyperinsulinemia.
7. Long chain polyunsaturated fatty acids (C20–C22) in muscle phospholipids are inversely related to hyperinsulinemia and positively related to insulin sensitivity.

of glucose into the cell is dependent on the binding of insulin to its receptors on muscle plasma membranes, which initiates a series of events that lead either to glucose oxidation or storage as glycogen. In normal subjects, most of the variance in insulin sensitivity is due to variations in glycogen synthesis.¹⁵ In patients with NIDDM, impairment in glycogen synthesis accounts for a major component of insulin resistance.¹⁶ Defects in glycogen synthesis predominate in the early stages of diabetes and have been reported in patients with increased risk of NIDDM. A recent report found an association between polymorphism of the glycogen synthase gene and NIDDM.¹⁷ The XbaI polymorphism of the glycogen synthase gene identifies a subgroup of patients with NIDDM, characterized by a strong history of NIDDM, a high prevalence of hypertension, and marked insulin resistance. A significant portion of the variance in insulin resistance seen from person to person is genetically determined.¹⁸ Insulin action can be modulated by environmental factors as shown in Table 1.

INSULIN RESISTANCE AND ASYMPTOMATIC ATHEROSCLEROSIS

The investigations by Laakso et al. provide the first direct evidence that asymptomatic atherosclerosis is associated with the etiology of insulin resistance.⁴ Metabolic studies indicate that insulin resistance is associated with asymptomatic atherosclerosis, even without significant hyperinsulinemia.⁴ These results suggest that the primary events responsible for atherosclerosis most likely are related to insulin resistance per se instead of the elevated insulin levels as suggested in previous studies.^{10–13,19–22} The impaired glucose uptake in peripheral tissues (muscle) is likely to be a primary phenomenon in atherosclerosis, and the elevation of insulin levels is likely to be the secondary mechanism. As shown by Laakso et al.²³ and Baron et al.,²⁴ in obesity and diabetes, respectively, reduced rates of glu-

Table 2. Metabolic Abnormalities Noted in Asymptomatic Atherosclerosis, Hypertension, Microvascular Angina, NIDDM, and Obesity

Insulin Resistance States	Abnormal Vascular Smooth Muscle Response	Whole Body Glucose Uptake	Glucose Nonoxidation (Glycogen Synthesis)	Glucose Oxidation	Lipid Oxidation	Suppression of Free Fatty Acids	Promotion of K Uptake During Hyperinsulinemia
Asymptomatic atherosclerosis	yes	reduced	reduced	← no difference between patients and controls →			
Hypertension	yes	reduced	reduced				
Microvascular angina	yes	reduced	← subtle changes →				
NIDDM	yes	reduced	reduced	reduced	reduced	reduced	reduced
Obesity	yes	reduced	reduced	reduced	reduced	reduced	reduced

cose uptake could be due to the decreased effect of insulin to stimulate microvascular blood flow in skeletal muscle and decreased delivery of glucose. Although blood flow was not measured in this study,⁴ it could be a factor.

INSULIN RESISTANCE AND MICROVASCULAR ANGINA

In about 20% of patients with angina-like chest pain, coronary angiography shows normal coronary arteries.²⁵ The results of the study by Botker et al. clearly establish that microvascular angina is a state of insulin resistance.⁴ The degree of insulin resistance is considerable in this entity, because the observed stimulation of whole body glucose uptake by insulin was about 40% less in the patients than in a well-matched control group,⁵ that is, an impairment of the same magnitude as found in essential hypertension,²⁶ NIDDM, and obesity.²⁷ Both oxidative glucose metabolism and nonoxidative glucose disposal rates are involved in NIDDM and obesity, but by contrast with findings in these disorders, patients with microvascular angina are characterized merely by subtle changes in endogenous glucose output and lipid metabolism. In terms of metabolic abnormalities, microvascular angina resembles more those of essential hypertension (Table 2). Furthermore, these results suggest that the metabolic disarray of microvascular angina is primarily confined to carbohydrate metabolism in skeletal muscle.

Patients with insulin resistance and microvascular angina develop atherosclerotic coronary artery disease only rarely, despite the hyperinsulinemia and modest dyslipidemia, suggesting that the insulin-resistance syndrome may represent a variety of disorders in which the endocrine defect only partly determines clinical expression. The data provide evidence that patients with microvascular angina are insulin-resistant independently of body mass index (BMI) and physical fitness. Botker et al. state that the concomitant occurrence of abnormal hyperemic response to ischaemia in the

forearm, oesophageal motility abnormalities, and irregular broncho-constrictor responses to methacholine inhalation further suggest the presence of a generalized disorder of smooth muscle.⁵ Abnormal vascular smooth muscle responses have also been reported in hypertension and NIDDM, conditions in which insulin resistance and subsequent hyperinsulinemia are common features.²⁸ Atherosclerosis, microvascular angina, and hypertension in patients with hyperinsulinemia may represent different entities of a more fundamental vascular disorder. Thus, insulin resistance syndromes share some defects but differ in others, suggesting that the underlying mechanisms must differ in part.

Table 2 is a summary of the metabolic abnormalities noted in asymptomatic atherosclerosis, hypertension, microvascular angina, NIDDM, and obesity. All these diseases have in common abnormal vascular smooth muscle response in addition to insulin resistance.

FATTY ACIDS AND INSULIN RESISTANCE: A HYPOTHESIS

Table 1 includes a number of environmental factors that influence insulin action. Diet is one factor of major importance. Both diet and physical activity influence insulin action. Saturated fats and alcohol increase insulin resistance, whereas physical activity decreases it. Dietary polyunsaturated fatty acids (PUFA) influence the level of both plasma and membrane phospholipid fatty acids. Complex interactions and displacements of the omega-3 and omega-6 fatty acids take place in plasma and cellular lipids after dietary manipulation. Early steps of cell activation, such as generation of inositol phosphate, indicate that diet-induced modifications of PUFA at the cellular level affect the activity of the enzymes responsible for the generation of lipid mediators in addition to the formation of products (eicosanoids) directly derived from their fatty acids precursors. This shows that dietary fats affect key processes in cell function.²⁹ Omega-3 fatty acids, especially the incorporation of docosahexaenoic acid

Table 3. Factors That Decrease C20-C22 Long Chain Polyunsaturated Fatty Acids (PUFA) in Muscle Phospholipids

Decreased intake of C20-C22
Increased linoleic acid (18:2w6) intake
Factors interfering with desaturation and elongation of 18:2w6 and 18:3w3 to C20-C22, such as increased trans fatty acid intake
Genetic defects in delta-6 and delta-5 desaturase
Increased catabolism of arachidonic acid (AA)

(DHA) into phospholipids, prevent the expected insulin resistance in rats fed a high fat diet.³⁰ Differences in the content of muscle membrane C20-C22 long chain PUFA have been shown to correlate with insulin sensitivity in human beings.³¹ Specifically, decreases in C20-C22 long chain PUFA were associated with increased insulin resistance. The amount of arachidonic acid (AA) in muscle membrane phospholipids was found to be inversely proportional to fasting insulin levels in patients with coronary artery disease.³¹ In normal individuals, AA and the total C20-C22 correlated positively with insulin sensitivity. Of interest in the Borkman study is the finding that in patients with coronary artery disease, linoleic acid (18:2w6) correlated directly with hyperinsulinemia, but not in normal controls.³¹ A recent study showed a direct correlation between dietary intake of 18:2w6 and coronary artery disease.³² Because PUFA intake influences insulin resistance, it is essential that we examine the factors that lead to a decrease of C20-C22 as well as an increase of linoleic acid in muscle membrane phospholipids (Table 3).

The current food supply is relatively low in AA, EPA, and DHA, whereas it is higher in 18:2w6 and relatively lower in 18:3w3 when compared to paleolithic nutrition on which humans evolved and for which humans were genetically programmed^{33,34} (Table 4). The reasons for the enormous increase of 18:2w6 are the indiscriminate use of vegetable oils and the recommendation to substitute margarines for saturated fats in the diet in the past 40–50 years.^{34–37} Margarines contain both 18:2w6 and trans fatty acids. Trans fatty acids interfere with the desaturation and elongation of 18:2w6 and 18:3w3, further contributing to decreases

in C20-C22 long chain PUFA.³⁸ In liver cell cultures, AA, eicosapentaenoic acid (EPA), and DHA decrease fatty acid synthase by decreasing its mRNA production.³⁹ A decrease in C20-C22 leads to increased fatty acid synthase, lipogenesis, fat deposition, and obesity. In rat studies, trans fatty acids increased 18:2w6, while lowering AA in tissue phospholipids, indicating inhibition of delta-6 desaturase. In these studies, there was an increase in fat cell size.³⁸

Kuller reported that women who consumed margarine four or more times per week weighed 2.3 kg more and had significantly lower HDL₂ and higher total cholesterol and triglycerides than women who consumed margarine less frequently.⁴⁰ The difference in weight between the two groups was unexplained because they reported similar energy intake and physical activity. In Kuller's study, the difference in weight is due most likely to the effect of trans fatty acids inhibiting desaturation and elongation of 18:2w6 and 18:3w3, resulting in lower levels of C20-C22 PUFAs in cell membranes with a subsequent increase in fatty acid synthase, leading to lipogenesis and obesity and/or to hyperinsulinemia, leading to decreased fatty acid oxidation, followed by lipogenesis and obesity.⁴¹ Siguel and Lerman determined plasma trans fatty acid levels in patients with angiographically documented coronary artery disease and concluded that dietary trans fatty acids are a cardiovascular disease risk factor.⁴²

Figure 1 presents a hypothetical scheme of how a decrease in C20-C22 from (1) a decrease in dietary intake per se, (2) increased dietary intake of trans fatty acids, (3) an increased intake of 18:2w6, or (4) genetic defects in delta-6 and delta-5 desaturase may lead to a decrease in C20-C22 muscle cell membrane phospholipids. Such a decrease leads to insulin resistance and hyperinsulinemia, with the subsequent development of obesity, hypertension, NIDDM, and coronary artery disease (including asymptomatic atherosclerosis and microvascular angina). Because all these conditions have strong genetic determinants, their development will depend on the interaction with environmental factors (diet, alcohol, physical activity).

CONCLUSION

Assuming that insulin resistance is the pathophysiologic component of the various mechanisms involved, it should be measured to identify those at risk and intervene. Because all obese individuals and NIDDM patients are insulin-resistant, there is no need to measure insulin resistance in them. Normal weight individuals with hypertriglyceridemia, low HDL, hypertension, coronary artery disease, and angina, as well as the offspring of diabetic and nonobese hypertensive

Table 4. Polyunsaturated Fatty Acid Content in Typical Western and Hunter-Gatherer Diets (g fatty acid type/person/day)

Diet	Linoleic and Linolenic Acids	Long Chain w6 and w3 PUFA	w6/w3 Ratio
Western diet	12.3	0.2	12
Hunter-gatherer diet	3.3	2.3	2.4

Modified from Ref. 33.

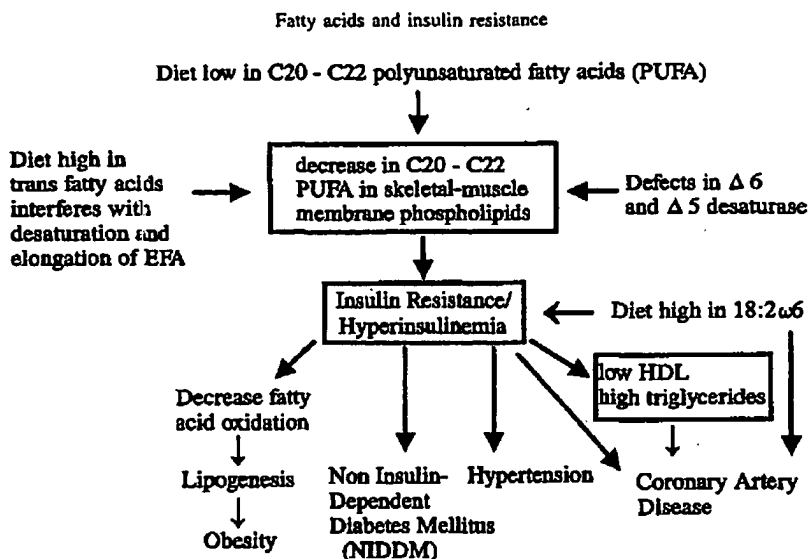


Fig. 1. A hypothetical scheme of the effects of dietary C20-C22 PUFA on the composition of the C20-C22 PUFA in skeletal-muscle membrane phospholipids and their relationship to insulin resistance/hyperinsulinemia and chronic diseases (obesity, NIDDM, hypertension, coronary artery disease).

parents and those with a family history of heart disease, should be checked for insulin resistance. Furthermore, because drugs are not yet available for treating insulin resistance, changes in diet and increase in energy expenditure should be recommended because weight loss and an increase in physical activity decrease insulin resistance. Interventional studies are needed to determine whether insulin resistance can be lowered through early treatment, by preventing the decrease in C20-C22 through a diet low in trans fatty acids, adequate in foods rich in AA (lean meat, fish, and eggs) and EPA and DHA (fish or fish oils), and balanced in 18:2ω6 and 18:3ω3 PUFA.

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The Influence of Diet on the Appearance of New Lesions in Human Coronary Arteries

David H. Blankenhorn, MD; Ruth L. Johnson, RD, MA; Wendy J. Mack, PhD; Hafez A. El Zein, MD; Laura I. Vailas, RD, MS

The Cholesterol Lowering Atherosclerosis Study, a randomized, placebo-controlled trial of blood lipid lowering, demonstrated significant benefit in 2-year coronary angiograms. Using angiograms of subjects in the Cholesterol Lowering Atherosclerosis Study who received a placebo and 24-hour dietary recall data, we performed an epidemiologic study of risk factors for formation of new atherosclerotic lesions. Age and baseline plus on-trial lipid levels, blood pressure levels, and diet variables were included. Significant dietary energy sources were protein, carbohydrate, alcohol, total fat, and polyunsaturated fat. Each quartile of increased consumption of total fat and polyunsaturated fat was associated with a significant increase in risk of new lesions. Increased intake of lauric, oleic, and linoleic acids significantly increased risk. Subjects in the Cholesterol Lowering Atherosclerosis Study in whom new lesions did not develop increased dietary protein to compensate for reduced intake of fat by substituting low-fat meats and dairy products for high-fat meats and dairy products. These results indicate that when total and saturated fat intakes are reduced to levels recommended by the National Cholesterol Education Program, protein and carbohydrate are preferred substitutes for fat calories, rather than monounsaturated or polyunsaturated fat.

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THE EVIDENCE for an association between diet and morbidity and mortality from coronary heart disease is substantial. The pioneering studies of Keys¹ in the Seven Countries Study demonstrated a significant association of high entry-level intake of dietary cholesterol and saturated fatty acids with high mortality rates from coronary heart disease at 5, 10, and 15 years.² At 15 years, mortality rates from coronary heart disease had a significant negative

correlation to the intake at baseline of monounsaturated fatty acids. Similarly, the Western Electric Study³ and the Ireland-Boston Diet-Heart Study⁴ found a positive correlation between Keys and Hegsted scores (measures of dietary intake of cholesterol and saturated and polyunsaturated fatty acids) and 19- and 20-year mortality rates from coronary heart disease, respectively. In addition, animal studies have shown clear evidence that dietary change influences progression and regression of atherosclerosis.^{5,6}

Severe restriction of caloric intake from all sources, including fat, can reduce coronary atherosclerosis. Diet de-

privation during World War I resulted in a reduction of atherosclerosis found at autopsy in Germany⁷ and World War II produced similar events in Scandinavia.^{8,9} Wilens¹⁰ reported reduced atherosclerosis in patients dying of wasting disease. However, a recent angiographic study, the Leiden Intervention Trial, provided evidence that a vegetarian diet that produced only minimal weight loss could reduce the progression of existing coronary atherosclerosis lesions in some patients.¹¹ The Leiden trial did not consider formation of new lesions, and there are no published data for humans on this subject.

We have previously reported that drug therapy with colestipol plus niacin in the University of Southern California's Cholesterol Lowering Atherosclerosis Study (CLAS) produced significant angiographic evidence of benefit in patients who had received aortocoronary bypass grafts.^{12,13} Dietary intervention in the CLAS consisted of a monitored reduction in the intake of fats and cholesterol while subjects consumed individually self-selected diets. Our angiographic assessment included evaluation of all lesions with greater than 20% diameter stenosis, and in a previous publication¹⁴ we identified 18 placebo recipients among a group of 82 in whom new native coronary artery lesions developed.

Two large autopsy studies have shown that the extent of coronary luminal surface covered by visibly raised lesions is a significant determinant of risk of death from myocardial infarction.^{15,16}

From the Atherosclerosis Research Institute, University of Southern California School of Medicine, Los Angeles.

Reprint requests to Atherosclerosis Research Institute, University of Southern California, RMR 102, 2025 Zonal Ave, Los Angeles, CA 90033 (Dr Blankenhorn).

1646 JAMA, March 23/30, 1990—Vol 263, No. 12

Diet and Coronary Lesions—Blankenhorn et al

In one of these,¹⁸ the extent of raised lesions was prime among significant coronary morphological determinants of risk for myocardial infarction. Since angiograms provide information about the extent of raised coronary lesions and we had obtained standardized 24-hour diet recall records during the CLAS, our placebo-treated subjects offered an opportunity for prospective observation of dietary effects (if any) on early changes in coronary artery anatomy that relate to prevention of myocardial infarction. We report herein a conventional epidemiologic evaluation of diet intake and change in disease status, in this case the presence or absence of new raised coronary lesions during a 2-year observation interval.

METHODS

Study Population

The CLAS was a randomized, placebo-controlled, angiographic trial that recruited nonsmoking men, aged 40 through 59 years, with progressive atherosclerosis who had undergone coronary bypass surgery. Screening procedures and criteria for exclusion, as well as standard visit protocols and intervention goals, were reported earlier.¹⁸ One hundred eighty-eight subjects were randomized into two equally sized treatment groups: 162 completed the study, 82 of these were placebo treated. Average age at entry of all placebo subjects was 54.5 ± 0.5 (SEM) years and average blood pressure was 125 ± 1.6 (SEM)/ 80 ± 1.0 mm Hg. Thirty percent had never smoked and 70% were ex-smokers for more than 6 months prior to trial entry. Twenty-four-hour urine specimens were covertly examined for nicotine and cotinine every 6 months and were positive in one subject once and one other subject twice in a 2-year period. Levels of physical activity were moderate, as judged by energy expenditure estimated by questionnaire and comparison of caloric intake recorded for 7 days before each clinic visit with body weights.¹⁸

Diet Goals

Dietary goals for the placebo-treated group were total fat calories to provide 28% of energy, less than 5% of energy from saturated fat, 10% of energy from polyunsaturated fat, 10% of energy from monounsaturated fat, and less than 250 mg of cholesterol per day. A comparison of the CLAS placebo group diet prescription with published National Cholesterol Education Program (NCEP) guidelines for step 1 and step 2 diet therapy¹⁹ appears in the "Comment" section.

Diet Counseling

We emphasized the importance of selecting an acceptable, affordable diet, compatible with each subject's life-style and culture. At an initial visit, the nutritionist explained the relationship between diet and blood lipid levels. At every visit during the trial, a computer printout of the nutrient content of food intake during the previous 7 days (recorded on mark-sensitive cards) was reviewed with each subject and discussed in relation to recent blood lipid levels. This approach was used to modify progressively the diet eaten by each subject to meet CLAS goals. Four optional weekly group counseling sessions that involved the subjects and their families were held soon after randomization. A bimonthly newsletter that contained nutrition information, fat-modified recipes, and an updated restaurant guide was sent to all subjects.

Diet Measurement

Standardized 24-hour dietary recalls were recorded by nutritionists certified by the University of Minnesota Nutrition Coordinating Center.^{20,21} A total of three 24-hour dietary recalls was scheduled for each subject: at baseline and the end of 1 and of 2 years in the trial. The recalls were processed by the Nutrition Coordinating Center for nutrient content and constituent food groups using the Nutrition Coordinating Center's database version 10.1.²² Major food groups were divided into animal, vegetable, and mixed sources of protein. Protein sources of animal origin were classified as follows: (1) low-fat meats (<10% fat) and high-fat meats ($\geq 10\%$ fat), (2) low-fat dairy products (<2% fat) and high-fat dairy products ($\geq 2\%$ fat), and (3) other protein sources (principally protein from eggs and egg whites).

Data Collection

Angiographic.—Baseline coronary angiography was performed immediately before randomization. A repeated angiogram was performed at 2 years by the same angiographer, who was blinded to the treatment assignment. Coronary angiogram evaluation procedures have been described previously in detail.^{18,19} All lesions with greater than 20% diameter stenosis were identified. Film pairs that showed identical coronary artery views were mounted side by side on two 35-mm film projectors (Vanguard Instrument Corp, Melville, NY) and viewed simultaneously by two expert angiographers who did not know the subject's demographic and clinical characteristics or treatment assignment or

the temporal order of the angiograms. They were not shown ventriculograms and other angiograms. Working independently, the two angiographers identified all visible lesions in one film (temporal order assigned at random), recorded this information, and then shared it to reach a consensus. Next, they examined the second film alongside the first and compared all lesions first separately and then in consensus. After all film pairs (plus a random 17% duplicate sample) were evaluated, the code for the film order was broken. Lesions recorded in a later angiogram but not in a first were considered new lesions. Thirteen among the 18 subjects with new lesions reported herein had a single new lesion, 3 had two new lesions, and 1 each had three and four new lesions. Fifty-seven percent of new lesions were in ungrafted patent arteries, 4% were proximal to complete occlusion in an ungrafted artery, 23% were proximal to open grafts, 8% were distal to open grafts, and 8% were distal to closed grafts. A new lesion in the distal circumflex coronary artery (ungrafted) is illustrated in the Figure.

Body Weight and Plasma Lipids.—Body weight and plasma lipid levels were measured at every visit.¹⁸ Baseline values are the mean of the first three screening visits. Postrandomization visits were scheduled every month for the first 6 months and bimonthly thereafter. On-trial values are the average of individual means weighted by the scheduled interval between treatment visits.

Dietary.—Baseline dietary nutrient data were obtained from the 24-hour dietary recall completed at entry. On-trial dietary nutrient data were the mean values for two recalls completed at the end of 1 and 2 years after randomization. Two subjects completed only one on-trial recall and values from this recall were used for them. The assumptions used to extrapolate overall consumption during the span of the CLAS from three standardized 24-hour recalls are modeled on those used for analysis of the Multiple Risk Factor Intervention Trial, although our study included a smaller number of study subjects.²¹ Comparison of caloric intake with weight change in our study suggested more complete recall than was obtained in the Multiple Risk Factor Intervention Trial. In the Multiple Risk Factor Intervention Trial, a 4-year average, 500-calorie deficit per day from baseline intake was not reflected in long-term weight change. In our study, all placebo recipients reported 10.7 kcal/lb per day at baseline and 10.9 kcal/lb per day during the trial. They showed no change in

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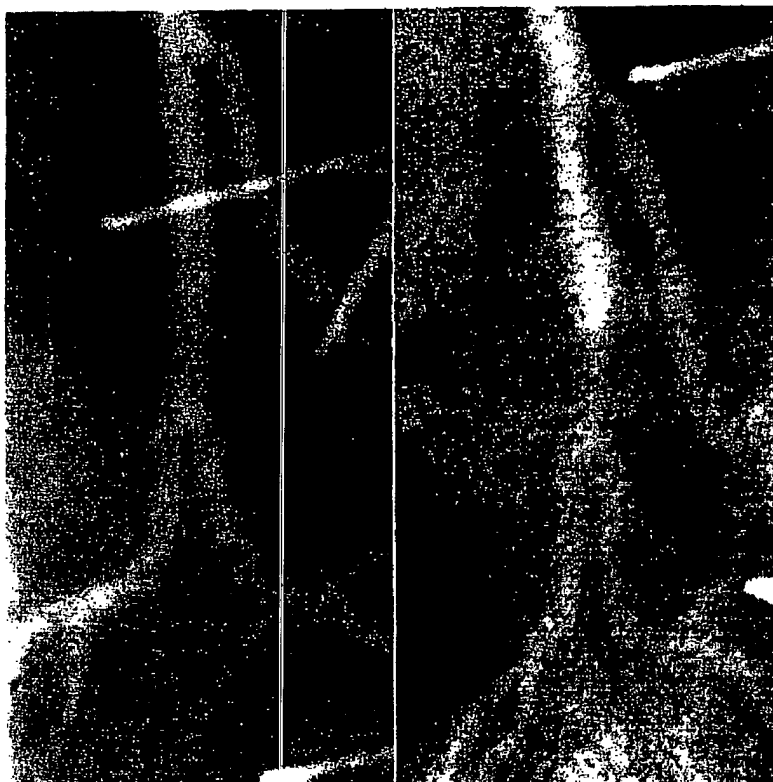
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JAMA, March 23/30, 1990—Vol 263, No. 12

Diet and Coronary Lesions—Blankenhorn et al 1647



A lesion of the distal circumflex coronary artery visible in 1998 (right) but not present in 1984 (left).

weight, and their reported caloric intake is compatible with estimates derived from resting metabolic rate measurements in sedentary men."

Statistical Methods

Univariate and multivariate logistic regression analyses were performed to determine which dietary variables were significantly associated with the development of new lesions. From these analyses, crude and adjusted odds ratios (baseline age, diet, and plasma cholesterol and blood pressure levels) and their associated 95% confidence intervals were determined. Dietary variables whose confidence intervals did not include 1.0 were considered to be significantly predictive of lesion development.

Stepwise logistic regression analyses were used to determine which dietary variables were independently significantly related to development of new lesions.

RESULTS

Baseline and on-trial dietary variables are summarized by lesion group in Table 1. Both groups reported lower intake of cholesterol and total fat at

baseline than was reported by free-living men of their age range in the National Health and Nutrition Evaluation Study II, where levels were 437 mg/d of cholesterol and 37.7% of energy from total fat.²⁴ Table 2 presents clinical variables.

Table 3 presents univariate logistic odds ratios and 95% confidence intervals for each dietary variable at baseline and during the trial. Each dietary variable was adjusted for age, systolic blood pressure, and total plasma cholesterol (Table 2). After this adjustment, no baseline dietary variable was significant. Mean on-trial protein (percent of energy) was significantly protective; those subjects not showing new lesions reported consumption of more protein during the trial than did subjects in whom new lesions developed. In contrast, mean on-trial higher percents of energy from total fat and polyunsaturated fat significantly increased the risk of new lesions developing.

On-trial food sources of protein were examined to determine which might be predictive of lesion development. Considering high-fat meat, low-fat meat, high-fat dairy products, low-fat dairy products, vegetable protein, mixed protein (animal plus vegetable), and miscel-

laneous protein (principally egg white) in logistic regression analyses, low-fat meat emerged as a significant protective factor (odds ratio, 0.83; 95% confidence interval, 0.69 to 1.00). In addition, the combination of low-fat meat and low-fat dairy products was significantly protective against lesion development (odds ratio, 0.82; 95% confidence interval, 0.69 to 0.96). No other protein sources were significantly associated with lesion development.

Trends in risk by increasing levels of mean on-trial fats were examined by dividing the entire subject group by quartiles according to on-trial fat consumption. In Table 4, an odds ratio of the lowest quartile of fat consumption set to 1 serves as a basis to compare odds ratios of risk in higher quartiles of fat consumption. Each quartile of increased consumption of total fat, saturated fat, and polyunsaturated fat is associated with increased risk; the trend is significant for total fat and polyunsaturated fat, confirming the results shown in Table 3. The 95% confidence limits of the odds ratios of highest quartile consumption of total fat, monounsaturated fat, and polyunsaturated fat all exceed 1.

Individual dietary fatty acids were analyzed to determine which, if any, specific fatty acids might be responsible for this association. Because of the relatively low levels of consumption of many fatty acids, quartiles of percent energy consumption for each fatty acid were computed using the entire sample of 82 subjects. Categories of consumption were dichotomized above and below the third quartile. After adjusting for plasma triglyceride levels, three on-trial fatty acids emerged as significant risk factors for development of new lesions. These were consumption of more than 0.22% of energy from lauric acid (17% of the group with no new lesions vs 60% of the group of subjects who developed new lesions [odds ratio, 4.56; 95% confidence interval, 1.45 to 14.32]), consumption of more than 11.33% of energy from oleic acid (19% of the group with no new lesions vs 44% of the group of subjects who developed new lesions [odds ratio, 3.27; 95% confidence interval, 1.05 to 10.22]), and consumption of more than 9.73% of energy from linoleic acid (20% of the group with no new lesions compared with 44% of the group of subjects who developed new lesions [odds ratio, 3.25; 95% confidence interval, 1.06 to 10.03]).

The relationship between dietary fat and plasma lipids was examined by comparing mean on-trial lipid levels between groups dichotomized at the median level of on-trial total fat, 29%

Table 1.—Mean Baseline and On-Trial Amounts of Nutrients for Subjects With New Lesions (n=18) and Without New Lesions (n=64)

	Baseline Amounts, Mean (SEM)		On-Trial Amounts, Mean (SEM)	
	No New Lesions	New Lesions	No New Lesions	New Lesions
Total kcal	1903.3 (85.7)	1903.9 (143.7)	1835.9 (53.1)	1853.0 (84.3)
Calories from protein, %	17.8 (0.6)	15.9 (1.1)	17.4 (0.4)	15.0 (0.6)
Calories from carbohydrate, %	45.2 (1.5)	52.8 (2.4)	52.3 (1.3)	50.8 (2.1)
Calories from total fat, %	32.9 (1.4)	31.1 (2.1)	27.5 (1.0)	34.1 (1.9)
Calories from saturated fat, %	11.3 (0.5)	10.7 (1.0)	7.4 (0.4)	9.2 (0.9)
Calories from polyunsaturated fat, %	6.7 (0.5)	6.1 (0.6)	8.6 (0.4)	10.9 (0.8)
Calories from monounsaturated fat, %	12.4 (0.6)	11.9 (1.0)	8.3 (0.4)	11.3 (1.0)
Polyunsaturated/saturated fat ratio	0.73 (0.07)	0.71 (0.13)	1.38 (0.07)	1.52 (0.19)
Calories from alcohol, %	5.5 (0.9)	1.8 (0.8)	5.0 (0.9)	3.0 (1.0)
Cholesterol, mg/dl	278.4 (27.8)	194.0 (37.2)	168.7 (15.5)	155.7 (28.3)
Calcium, mg/d	859.2 (61.6)	890.5 (87.1)	950.8 (48.6)	828.8 (62.1)

Table 2.—Mean Baseline and On-Trial Clinical Measures for Subjects With New Lesions (n=18) and Without New Lesions (n=64)

	Baseline Measures, Mean (SEM)		On-Trial Measures, Mean (SEM)	
	No New Lesions	New Lesions	No New Lesions	New Lesions
Relative weight	1.19	1.18	1.19	1.18
Weight, lb	177.7 (2.9)	178.3 (3.9)	176.8 (3.1)	178.1 (3.8)
kcal/lb	10.7	10.7	10.4	11.0
Total cholesterol, mg/dL*	243 (4)	242 (9)	230 (4)	240 (11)
LDL† cholesterol, mg/dL	170 (4)	165 (7)	158 (3)	164 (9)

*To convert cholesterol in milligrams per deciliter to millimoles per liter, multiply by 0.02586.
 †LDL indicates low-density lipoprotein.

Table 3.—Univariate Dietary Intake Associations With New Lesion Development*

	Baseline Associations		Mean On-Trial Associations	
	OR	95% CI	OR	95% CI
Total kcal	1.00	1.00-1.00	1.00	1.00-1.00
Calories from protein, %	0.94	0.82-1.08	0.72	0.57-0.92
Calories from carbohydrate, %	1.05	1.00-1.11	0.98	0.82-1.04
Calories from total fat, %	0.97	0.92-1.03	1.11	1.02-1.20
Calories from saturated fat, %	0.95	0.84-1.09	1.13	0.95-1.35
Calories from polyunsaturated fat, %	0.93	0.77-1.11	1.28	1.07-1.53
Calories from monounsaturated fat, %	0.96	0.85-1.08	1.15	0.97-1.38
Calories from alcohol, %	0.92	0.80-1.06	0.97	0.87-1.08
Cholesterol, mg/d	1.00	0.99-1.00	1.00	0.99-1.00
Calcium, mg/d	1.00	1.00-1.00	1.00	1.00-1.00

*OR indicates odds ratio; and 95% CI, 95% confidence interval. The OR represents change in risk for lesion development per unit change of a given variable. For example, a subject's risk increases by a multiplicative factor of 1.11 for each increase of 1% of calories from mean on-trial total fat.

of calories. Total plasma cholesterol levels were significantly different ($t=2.01$; $P=.048$) between the two dietary fat groups. At or below the median level of dietary fat, the mean total plasma cholesterol level was 224.5 ± 4.9 (SEM) mg/dL (5.80 ± 0.15 mmol/L); above the median level of dietary fat, the total cholesterol level was 240.1 ± 6.1 mg/dL (6.20 ± 0.15 mmol/L). Levels of plasma triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and non-high-density lipoprotein cholesterol

were not significantly different between the two dietary fat intake groups.

To determine if a relatively short-term (2-year) change in dietary habits can influence the development of new lesions, logistic regression analyses were performed on dietary change variables (baseline minus on-trial values). To adjust for any differences in baseline dietary levels between the two lesion groups that might confound the relationship between dietary change and lesion development, baseline values for each dietary variable were also included

in the logistic regression model. Table 5 presents the results of summary data for dietary change as well as the results of the logistic regression analyses. The group of subjects who developed new lesions showed a larger mean decrease in percent of energy intake from proteins, while those subjects without new lesions increased mean protein intake. Decrease in protein intake was a significant risk factor for lesion development. The decreased percents of energy from total, polyunsaturated, and monounsaturated fat were significant protective factors. In this context, the group in whom new lesions did not develop showed a mean decrease in total fat intake, while the group in whom lesions did develop had an overall mean increase in total fat. Although both groups increased their mean percent intake from polyunsaturated fats, this increase was larger for the group of subjects who developed new atherosclerotic lesions. In addition, decreases in mean percent of calories from monounsaturated fats were larger for the group in whom new lesions did not develop.

Table 6 presents results of two separate stepwise logistic regression analyses. In the first model, with all variables competing, greater percent of energy from mean on-trial protein, greater on-trial decrease in total dietary fat, and greater age and alcohol intake at study entry emerged as independently significant protective factors. In the second stepwise analysis, the variable total fat at baseline and during the trial was not allowed to compete in the stepwise model, but all other variables, including saturated, polyunsaturated, and monounsaturated fat variables, were allowed to compete. Results of this analysis indicate that greater percent of energy from mean on-trial protein, greater baseline alcohol intake, on-trial increase in carbohydrate intake, and decrease in polyunsaturated fat intake were independently protective.

COMMENT

The majority of CLAS placebo recipients ($n=64$) did not develop new lesions during a 2-year observation interval, but there were 18 subjects who did, and we applied a traditional epidemiologic approach to evaluate dietary differences between the two groups. We classified subjects with new lesions as "diseased" and those without as "nondiseased" and computed risk for development of new lesions after baseline differences in age, blood pressure, blood lipid levels, and diet were taken into account. Our analysis adjusted for short-term differences between sub-

Table 4.—Relative Risks for Lesion Development by Quartiles of Mean On-Trial Dietary Fats*

	No. of Patients With New Lesions	No. of Patients Without New Lesions	OR	95% CI
Calories from total fat, %				
0-23.0	1	20	1.00	...
>23.0-29.0	4	16	5.00	0.51-49.27
>29.0-33.9	5	15	6.67	0.71-63.18
>33.9	8	13	12.31	1.37-110.31
Calories from saturated fat, %				
0-5.2	3	18	1.00	...
>5.2-7.1	3	17	1.06	0.19-5.98
>7.1-10.1	5	16	1.87	0.39-9.12
>10.1	7	13	3.23	0.70-14.91
Calories from polyunsaturated fat, %				
0-6.8	1	20	1.00	...
>6.8-8.6	3	17	3.53	0.83-37.15
>8.6-11.1	6	14	8.57	0.93-79.27
>11.1	8	13	12.31	1.37-110.31
Calories from monounsaturated fat, %				
0-8.9	3	18	1.00	...
>8.9-9.8	5	15	2.00	0.41-9.78
>9.8-12.3	1	20	0.30	0.03-3.15
>12.3	9	11	4.91	1.09-22.15

*OR indicates odds ratio; and 95% CI, 95% confidence interval.

Table 5.—Associations Between Change in Dietary Intake and the Development of New Lesions*

	Mean Change (SEM)		OR	95% CI
	No New Lesions	New Lesions		
Total kcal	87.5 (85.4)	-49.1 (171.4)	1.00	1.00-1.00
Calories from protein, %	-0.4 (0.6)	1.0 (1.0)	1.37	1.08-1.75
Calories from carbohydrate, %	-7.1 (1.6)	2.3 (2.5)	1.05	0.98-1.12
Calories from total fat, %	5.3 (1.6)	-3.0 (2.5)	0.90	0.83-0.98
Calories from saturated fat, %	3.9 (0.7)	1.5 (1.1)	0.88	0.75-1.04
Calories from polyunsaturated fat, %	-1.9 (0.8)	-4.8 (1.0)	0.78	0.65-0.93
Calories from monounsaturated fat, %	3.1 (0.8)	0.7 (1.0)	0.86	0.73-1.03
Calories from alcohol, %	0.5 (1.0)	-1.2 (1.2)	1.01	0.91-1.12
Cholesterol, mg/d	111.7 (33.0)	38.3 (39.3)	1.00	1.00-1.01
Calcium, mg/d	-91.4 (67.8)	-138.3 (83.6)	1.00	1.00-1.00

*OR indicates odds ratio; and 95% CI, 95% confidence interval. Dietary change variables were computed as baseline minus mean on-trial value. Thus, an OR less than 1.00 means that a decrease in this dietary variable is a protective factor, and an OR greater than 1.00 means that a decrease in the dietary variable is a risk factor for lesion development.

Table 6.—Stepwise Logistic Regression Results*

	OR	95% CI	P
Analysis 1			
Calories from mean on-trial protein, %	0.70	0.54-0.90	.012
Calories from baseline alcohol, %	0.82	0.69-0.97	.019
Change in % of calories from total fat	0.92	0.87-0.98	.014
Age at study entry	0.88	0.74-1.00	.05
Analysis 2			
Calories from mean on-trial protein, %	0.70	0.54-0.90	.006
Calories from baseline alcohol, %	0.80	0.67-0.96	.017
Change in % of calories from carbohydrates	1.06	1.00-1.12	.041
Change in % of calories from polyunsaturated fat	0.65	0.73-0.89	.042

*OR indicates odds ratio; and 95% CI, 95% confidence interval. Variables are listed in the order entered into the stepwise model.

jects at trial entry, but not for long-term factors, except age. The significant protective effect of age (odds ratio, 0.86; 95% confidence interval, 0.74 to 1.00), a result of the CLAS entry requirement

for previous aortocoronary bypass grafting, will be discussed in a separate article. Both the number of subjects and diet recall records are limited, but angiographic trials typically involve fewer

subjects than coronary event-based trials, and this is the first information, to our knowledge, on dietary effects on the appearance of new coronary lesions in man.

Placebo recipients in the CLAS were at high risk for developing new coronary atherosclerotic lesions because they had prior aortocoronary bypass surgery.²² They also had blood levels of total cholesterol (243 ± 36 mg/dL [6.30 ± 0.95 mmol/L]) and low-density lipoprotein cholesterol (169 ± 31 mg/dL [4.35 ± 0.80 mmol/L]) at baseline just above the cut point designated as high risk by the NCEP.¹⁹ On entry, they reported an average diet intake that contained less fat and cholesterol than is average for Americans,²³ but higher in total fat and saturated fat than is recommended by the NCEP. The NCEP's recommendations are compared with CLAS baseline and on-trial intakes in Table 7. In the CLAS, on-trial intake of fat varied from an average of 34.1% of energy among those who developed new lesions to 27.5% of energy among those who did not. The median level of total fat intake was 29% of total calories, and subjects who ate more than this amount had significantly higher total plasma cholesterol levels than subjects whose fat intake was less than median.

Energy sources significantly influencing the appearance of new lesions in the CLAS were protein, carbohydrate, alcohol, total fat, and polyunsaturated fat. These are all closely linked, because subjects eating less calories from one source obtained more calories from another and the average weight of CLAS subjects did not change during the trial (Table 2). When all significant factors are considered together, the major sources of energy form a coherent picture consistent both with concepts derived from the epidemiology of coronary heart disease and with findings from the National Diet-Heart Study.²⁴ In accord with previous epidemiologic research,^{25,26} CLAS subjects who did not develop new lesions drank more alcohol at baseline (in moderate amounts) than those subjects who did develop new lesions. In accord with National Diet-Heart Study findings, CLAS subjects who did not develop new lesions increased on-trial dietary protein to compensate for reduced intake of fat by substituting low-fat meats and dairy products for high-fat meats and dairy products.

Diets that substituted low-fat for high-fat meats and dairy products were rigorously tested under randomized, double-blind conditions in the National Diet-Heart Study, in which fat intake at entry of 40% of calories was reduced to 30%.²⁴ In the National Diet-Heart

Table 7.—Dietary Intake by CLAS Placebo Recipients at Baseline and During the Trial Compared With NCEP Step 1 and Step 2 Dietary Therapy*

Nutrient	CLAS				
	NCEP		During Trial		
	Step 1	Step 2	Baseline	New Lesions	No New Lesions
Total fat, % calories†	<30	<30	32.2	34.1	27.5
Saturated fat, % calories	<10	<7	11.1	9.2	7.4
Monounsaturated fat, % calories	≤10	≤10	6.5	10.9	8.8
Polyunsaturated fat, % calories	10-15	10-15	12.2	11.3	9.3
Carbohydrates, % calories	50-60	50-60	47.2	50.6	52.3
Protein, % calories	10-20	10-20	17.4	15.0	17.4
Cholesterol, mg/d	<300	<200	258.7	155.7	166.7

*CLAS indicates Cholesterol Lowering Atherosclerosis Study; and NCEP, National Cholesterol Education Program.

†Percent calories represents percent of energy from nutrient.

Study, an 11% reduction in total plasma cholesterol level was maintained for 66 weeks. Intake of subjects in the National Diet-Heart Study provided adequate amounts of all essential nutrients. Placebo recipients in the CLAS also maintained adequate intake of all essential nutrients and showed increased intakes of calcium and retinol (data not shown), a result of increased intake of nonfat milk. Compared with whole milk, the calcium content of nonfat milk is not reduced and retinol content is increased by fortification.

Average total plasma cholesterol reduction in all CLAS placebo recipients (4.5%) was not as great as in the National Diet-Heart Study. However, the differential reduction in low-density lipoprotein cholesterol levels in CLAS subjects with and without new lesions was comparable with the differential in cholestyramine-induced low-density lipoprotein cholesterol reduction between men with and without ischemic heart disease in the Lipid Research Clinics Coronary Primary Prevention Trial.²⁴ Average low-density lipoprotein cholesterol level was reduced 7.1% in CLAS subjects who did not develop new lesions and 0.6% in those who did develop new lesions (Table 2), a significant differential of 6.5% ($P=.04$). After 2 years in the Lipid Research Clinics Coronary Primary Prevention Trial, cholestyramine-treated men without new ischemic heart disease showed a 21% reduction, those with new ischemic heart disease a 16.9% reduction; thus, there was a differential of 4.1%.

Fatty acids significantly increasing the likelihood of the appearance of new lesions were lauric, oleic, and linoleic. Lauric acid, a saturated C-12 fatty acid, is a major component of coconut and palm kernel oils. It is the shortest dietary fatty acid stored in the adipose tissue of humans and other species.^{24,25} Unfortunately, lauric acid was not in-

cluded in recent epidemiologic studies of blood and tissue fatty acids and incidence of ischemic heart disease.^{26,27} Hegsted et al²⁸ demonstrated in humans that for levels of dietary cholesterol between 200 and 600 mg/d, coconut oil produces greater increases in total plasma cholesterol level than olive or safflower oil. Coconut oil has also been found particularly effective in raising plasma and aortic cholesterol content for experimental atherosclerosis in cholesterol-fed rabbits.²⁹ In perfused rat liver preparations, short-chain saturated fatty acids lead to production of cholesterol-rich, potentially atherogenic very-low-density lipoprotein cholesterol.³⁰

Dietary oleic and linoleic acids are stored in adipose tissue and appear in atherosclerotic plaques as cholesterol esters.³¹ Three epidemiologic studies have suggested an inverse relationship between linoleic acid and risk of coronary heart disease,^{32,37,38} an effect confounded with risk from smoking in one.³⁹ In the CLAS, the likelihood of new lesions developing increased significantly with each quartile of increasing consumption of total fat, monounsaturated fat, and polyunsaturated fat, and linoleic acid intake was significantly associated with formation of new lesions. The CLAS subjects were nonsmokers and had reduced total and saturated fat intake before study entry to levels close to NCEP recommendations. Comparison of fat intakes within the CLAS is not equivalent to comparisons made across human populations with high and low intakes of dietary fat. It is also possible that the effects of transport and metabolism of unsaturated fatty acids are different in early stages of atherosclerosis as compared with late, because dietary fatty acids can influence a variety of arterial wall processes, ranging from endothelial permeability⁴⁰ to thrombosis and inflammatory responses.⁴¹ Reevaluation of traditional views on dietary fat

and coronary heart disease has recently been recommended by Oliver.⁴² However, all three dietary fatty acids—lauric, oleic, and linoleic acids—are transported in triglyceride-rich lipoproteins and influence their composition. The significant relationships found between these fatty acids and formation of new coronary lesions is compatible with a previous risk factor analysis based on lipoprotein, cholesterol, and apolipoprotein patterns in CLAS subjects.⁴³ This analysis indicated an important role for triglyceride-rich lipoproteins in human atherosclerosis. Taken as a whole, CLAS results indicate that when total and saturated fat intakes are reduced to NCEP-recommended levels, protein and carbohydrate are preferred substitutes for fat calories, rather than monounsaturated or polyunsaturated fat.

Reports of the reduction in arterial lesions at autopsy following semistarvation conditions during World Wars I and II have suggested the necessity of austere diets to ameliorate atherosclerosis. However, more reasonable alterations of diet appear adequate to produce detectable improvement of coronary lesions in angiographic studies. The Leiden Dietary Intervention Trial showed that existing atherosclerotic lesions in some patients can be stabilized by a diet that results in only minimal weight loss. Placebo recipients in the CLAS provide evidence that the appearance of new coronary atherosclerotic lesions can be influenced without weight change by voluntary selection of acceptable foods. Principally, this involved substitution of low-fat meats and dairy products for high-fat meats and dairy products. The diets eaten by CLAS subjects were self-selected, with monitoring and counseling by registered dietitians. They do not appear too stringent for wide application as a precaution against development of new coronary lesions.

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Can linoleic acid contribute to coronary artery disease?¹⁻³

Jonathan M Hodgson, Mark L Wahlqvist, John A Boxall, and Nicholas D Balazs

ABSTRACT The adipose tissue concentration of linoleic acid was positively associated with the degree of coronary artery disease (CAD) in a cross-sectional study of 226 patients undergoing coronary angiography. Linoleic acid concentration in adipose tissue is known to reflect the intake of this fatty acid. These results are therefore indicative of a positive relationship between linoleic acid intake and CAD. The platelet linoleic acid concentration was also positively associated with CAD. After confounding factors were allowed for, the eicosapentaenoic acid concentration in platelets was inversely associated with CAD for men, and the docosapentaenoic acid concentration in platelets was inversely associated with CAD for women; results consistent with several other studies that suggest that fish, and ω -3 fatty acids derived from fish and fish oils, can beneficially influence macrovascular disease. *Am J Clin Nutr* 1993;58:228-34.

KEY WORDS Linoleic acid, ω -3 fatty acids, polyunsaturated fatty acids, coronary artery disease, atherosclerosis

Introduction

Many studies have examined the relationships between diet and end points of coronary heart disease (CHD) such as angina, myocardial infarction, sudden death, angiographically assessed coronary artery disease (CAD), and coronary mortality. The majority have focused on the lipid components of the diet. A high intake of saturated fatty acids is now considered to be a positive risk factor for CHD and an adequate intake of ω -3 fatty acids is believed to be influential in preventing CHD. The role of linoleic acid (18:2n-6), an ω -6 essential fatty acid, however, is less clear.

The measurement of linoleic acid in adipose tissue provides a good estimate of long-term intake of linoleic acid (1, 2). Several studies have demonstrated an inverse relationship between adipose tissue linoleic acid content and CHD (3-6). Population studies have shown that low concentrations of adipose tissue linoleic acid are associated with increased rates of CHD (3, 4), and that this inverse relationship exists both between and within populations (4). A cross-sectional survey of Scottish men demonstrated that those with previously unidentified CHD, as defined by angina pectoris or myocardial infarction, had a significantly lower concentration of adipose tissue linoleic acid than did men without CHD (5). The inverse relationship between adipose tissue linoleic acid and angina pectoris or myocardial infarction has also been demonstrated in a case-control study (6). These results are supported by the observation that as the vegetable-animal fat ratio increased in Australia and North America, there has

been an associated reduction in total mortality. In England and Wales where minimal changes in this ratio occurred, there were also minimal changes in coronary mortality (7). However, not all studies have produced results that would indicate a protective role for linoleic acid. In a study by Blankenhorn et al (8) it was found that increased intake of linoleic acid significantly increased the risk of new atherosclerotic lesions in human coronary arteries.

Evidence for an inverse association between the long-chain ω -3 fatty acids or fish intake and CHD has been accumulating. A reduction in total mortality was demonstrated in a secondary prevention intervention study in which the intervention was fatty fish (9). Prospective studies have found an inverse association between fish intake and CHD incidence (10, 11), although an inverse association has not been demonstrated in all prospective studies (12, 13). Fish intake has also been associated with improved arterial wall characteristics (14). In a study in which platelet fatty acids were measured, eicosapentaenoic acid (20:5n-3) was inversely associated with angina pectoris and docosapentaenoic acid (22:5n-3) was inversely associated with risk of acute myocardial infarction. Also in this study, adipose tissue docosahexaenoic acid (22:6n-3) was inversely associated with acute myocardial infarction (6).

In our study the fatty acids in adipose tissue and platelets were measured. The relationships between each of the fatty acids and the degree of angiographically assessed CAD were examined.

Subjects and methods

Population sample

Some of the subject characteristics are given in Table 1. All patients underwent coronary angiography and were on the routine cardiac catheterization list for investigation of chest pain thought to be due to either CAD (97%) or valvular heart disease (3%). Consecutive patients (160 males and 66 females aged 16-80 y) were enrolled over 10 mo. All patients were included. The

¹ From the Departments of Medicine, Cardiology, and Clinical Biochemistry, Monash Medical Centre, Melbourne, Australia.

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³ Address reprint requests to ML Wahlqvist, Professor and Chairman of Medicine, Monash University Department of Medicine, Monash Medical Centre, Clayton Road, Clayton, Melbourne, Victoria 3168, Australia.

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LINOLEIC ACID AND CAD

229

TABLE I
Patient characteristics

Characteristic	Men (n = 160)	Women (n = 66)
	%	
Obtained from medical records		
Angina	98	95
Previous myocardial infarction	39	27
Valvular heart disease	2.0	8.2
Hypertension	41	63
Taking medication		
Aspirin	67	49
Nitrates	58	44
Calcium antagonists	48	42
Beta-blockers	41	39
Angiotensin-converting-enzyme inhibitors	16	29
Obtained from questionnaire		
Smokers	14	11
Exsmokers	58	58
Never smoked	28	31
Diabetes mellitus	8.7	9.1
No dietary change	49	44
Dietary change	51	56

project was presented to and approved by the Monash Medical Centre Ethics and Research Committee.

Clinical details such as age, diabetic status, smoking history, height, weight, and past dietary change were gathered by using a questionnaire administered at the time of angiography. Self-reported height and weight were used to calculate body mass index (BMI), which was calculated by dividing weight (kg) by the square of height (m). Information about dietary change was obtained with the questionnaire. Each patient was asked whether they had made a conscious decision to make dietary changes at any time in the previous 10 y. For analysis the patients were divided into two groups: no dietary change and dietary change. To estimate the total number of cigarettes smoked, the number of cigarettes smoked per day was multiplied by the duration of time that the patient smoked. The measurement of blood pressure was not included in this study for several reasons. Almost all hypertensive patients were being treated for hypertension. However, the anti-hypertensive drug treatment differed and so too did the duration of treatment and the degree of blood pressure lowering. This would make the interpretation of results from a single blood pressure measurement difficult. Information about history of hypertension and use of particular medication was collected from the medical records. The presence or absence of hypertension and use of particular medications were included in the analysis. Coronary angiography was performed according to the Judkins technique (15) and recorded on 35-mm movie film. Two different scoring systems were used to quantify the degree of CAD: a myocardial score and an extent score.

Angiographic scores

Myocardial score. A myocardial scoring system that takes into account the degree of stenosis of any number of arterial branches and their relative importance in terms of the amount of myocardium supplied, has been developed (16). This scoring system

takes into account the severity as well as the location of the coronary lesions. A score from 0 to 15 (best to worst condition) can be given.

Extent score. An angiographic scoring system that has been designed to reflect the proportion of the coronary endothelial surface area affected by atheroma has been developed as an estimate of the extent of coronary atherosclerosis (17). The proportion of the coronary arterial tree with detectable atheroma, identified as luminal irregularity, was scored with a maximum of 10; a score of 0 indicates that no coronary atheroma was detected, and a score of 10 means that 100% of the coronary arteries visualized showed detectable atheroma.

Serum lipid measurements

Fasting blood was drawn from the femoral artery immediately before cardiac catheterization and placed into evacuated glass tubes. The untreated blood was allowed to clot and the serum was separated by using standard procedures. Total cholesterol, triglycerides, and high-density-lipoprotein (HDL) cholesterol were measured in fresh serum.

Total cholesterol and triglycerides were measured enzymatically with commercial kits (13225 and 22203, respectively; Trace Scientific Pty Ltd, Clayton, Victoria, Australia). HDL cholesterol was measured enzymatically as for total cholesterol, after the precipitation of apolipoprotein B-containing lipoproteins by using equal volumes of 20% polyethylene glycol 6000 (Merck-Schuchardt, Munich, Germany) and serum. Low-density-lipoprotein (LDL) cholesterol was derived by using the Friedewald formula adapted to Système International (SI) units (18). Cholesterol and triglyceride measurements were performed on a KONE Progress selective chemistry analyzer (KONE Instruments Corporation, Espoo, Finland).

Fatty acid analysis

The fatty acid compositions of subcutaneous adipose tissue and platelets were measured by using gas chromatography. The methods relating to these measurements are presented below.

For collection and preparation of adipose tissue, ~1–2 mg adipose tissue was taken from the site of catheter insertion immediately after coronary angiography. The sample was frozen at -70°C for up to 12 mo, until all samples had been collected.

For collection and preparation of platelets, 20 mL EDTA-anticoagulated blood was drawn from the femoral artery immediately before cardiac catheterization and was used for platelet harvesting. The tubes were mixed immediately after blood collection and again before platelet harvesting. Tubes of blood were centrifuged at $110 \times g$ for 15 min and the platelet-rich plasma was removed then recentrifuged at $2000 \times g$ for 10 min at room temperature. The plasma was removed and the platelets were washed twice with 0.9% NaCl containing 1 g EDTA/L. The platelet pellet was then frozen at -70°C until extraction and methylation.

Extraction and methylation of the adipose tissue and platelet fatty acids were performed by using a modification of the one-step method described by Lepage and Roy (19). Samples were placed into glass tubes with 2 mL of 4:1 methanol:toluene and 200 μL acetyl chloride was added slowly to each tube with continuous mixing, then samples were placed into an oven at 100°C for 1 h. The tubes were cooled under running water, 2.5 mL potassium carbonate was added, and the tubes were mixed and then centrifuged at $2000 \times g$ for 10 min at room temperature.

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230

HODGSON ET AL

TABLE 2
Descriptive statistics for the study population*

Characteristic	Men	Women
Extent score (10)†	4.3 ± 1.9 [159]‡	3.4 ± 2.3 [66]
Myocardial score (15)§	7.7 ± 3.6 [159]‡	6.4 ± 4.25 [66]
Age (y)	59 ± 10.7 [159]	61 ± 10.3 [66]
Body mass index	25.7 ± 2.59 [132]	25.2 ± 4.30 [57]
Total cholesterol (mmol/L)	5.8 ± 0.93 [159]	6.4 ± 1.1 [66]
LDL cholesterol (mmol/L)	4.0 ± 0.86 [155]	4.4 ± 1.0 [66]
HDL cholesterol (mmol/L)	0.98 ± 0.28 [155]	1.25 ± 0.42 [66]
Triglycerides (mmol/L)	1.8 ± 0.96 [159]	1.7 ± 0.77 [66]
Cigarettes smoked ¶	741 ± 760 [129]	344 ± 606 [57]

* $\bar{x} \pm SD$; *n* in brackets.

† Scoring system for extent of coronary atherosclerosis, from 0 to 10.

‡ Significantly greater than women $P < 0.0001$.

§ Scoring system for degree of arterial stenosis, from 0 to 15.

|| In kg/m².

¶ Estimate of the total number of cigarettes smoked over a lifetime.

The upper toluene phase was removed and placed into small glass tubes, then dried under nitrogen.

Immediately after extraction and methylation of the fatty acids the methylated fatty acids were dissolved in 50 μ L chloroform for injection. A Shimadzu GC-9A gas chromatograph was used with a flame ionization detector and a Shimadzu Chromatopac C-R3A integrator (Shimadzu Corporation, Kyoto, Japan). A 50-m glass capillary column with an 80% cyanopropyl silicone polar phase was used. The methyl ester peaks were identified with standards obtained from Nu Chek Prep, Inc (Elysian, MN). Temperature programming was used for determining fatty acids. The starting temperature of 130 °C was raised at 5 °C/min until 190 °C was reached, then increased more slowly at 1.2 °C/min until 200 °C was reached. The temperature was then held constant at 200 °C for 15 min. The CV in determining the percentages of the individual fatty acids varied depending on the concentration of the fatty acid in the sample. For major components (> 5%) the CV ranged from 1.2% to 4.7%. For trace fatty acids (< 1%) the CV ranged from 7.4% to 14%.

Statistics

The data-analysis package used for all the statistical analyses performed was SAS (20,21). At a univariate level, Spearman's rank correlation coefficient (*r*) was used to determine the degree and direction of association between two variables. To control for covariates, the PARTIAL option was used. The Wilcoxon rank-sum test was performed to test whether there were differences between two population means.

Results

Descriptive statistics

Some descriptive statistics for the study population are presented in Table 2. The extent score ranged from 0 to 8.5 and was significantly higher for men than for women. The myocardial score ranged from 0 to 15 and was also significantly higher for men than for women. Nineteen patients (8.4%) had no detectable CAD, with both extent and myocardial scores of 0. The degree of correlation between the two scores was high ($r = 0.72$, P

TABLE 3
Fatty acid composition of adipose tissue and platelets*

Fatty acid	Men	Women
% of total fatty acids		
Adipose tissue fatty acids		
Lauric (12:0)	0.43 ± 0.28 [106]	0.38 ± 0.17 [39]
Myristic (14:0)	2.95 ± 0.80 [106]	2.67 ± 0.67 [39]
Palmitic (16:0)	23.1 ± 1.96 [106]	22.4 ± 2.47 [39]
Stearic (18:0)	4.47 ± 1.29 [104]	4.27 ± 1.39 [39]
Palmitoleic (16:1n-7)	5.87 ± 2.37 [106]	5.72 ± 2.25 [39]
Oleic (18:1n-9)	42.6 ± 3.11 [104]	42.5 ± 2.62 [39]
Linoleic (18:2n-6)	12.6 ± 3.82 [106]	13.7 ± 3.83 [39]
Homo- γ -linolenic (20:3n-6)	0.17 ± 0.05 [105]	0.25 ± 0.11 [39]
Arachidonic (20:4n-6)	0.32 ± 0.11 [105]	0.41 ± 0.12 [39]
Alpha-linolenic (18:3n-3)	0.56 ± 0.17 [106]	0.61 ± 0.23 [39]
Eicosapentaenoic (20:5n-3)	0.10 ± 0.05 [68]	0.11 ± 0.04 [26]
Docosapentaenoic (22:5n-3)	0.15 ± 0.06 [104]	0.20 ± 0.08 [39]
Docosahexaenoic (22:6n-3)	0.11 ± 0.06 [101]	0.18 ± 0.10 [39]
Platelet fatty acids		
Palmitic (16:0)	17.9 ± 1.23 [135]	18.2 ± 1.52 [55]
Stearic (18:0)	17.9 ± 1.11 [135]	17.7 ± 1.18 [55]
Palmitoleic (16:1n-7)	1.58 ± 0.37 [134]	1.61 ± 0.36 [55]
Oleic (18:1n-9)	15.7 ± 1.41 [135]	15.8 ± 1.22 [55]
Linoleic (18:2n-6)	5.46 ± 1.56 [135]	5.42 ± 1.07 [55]
Homo- γ -linolenic (20:3n-6)	0.98 ± 0.23 [134]	1.01 ± 0.21 [55]
Arachidonic (20:4n-6)	16.9 ± 1.50 [135]	16.7 ± 1.14 [55]
Docosatetraenoic (22:4n-6)	1.49 ± 0.36 [135]	1.51 ± 0.31 [55]
Alpha-linolenic (18:3n-3)	0.39 ± 0.16 [129]	0.37 ± 0.13 [49]
Eicosapentaenoic (20:5n-3)	2.14 ± 0.31 [135]	1.04 ± 0.35 [55]
Docosapentaenoic (22:5n-3)	0.86 ± 0.20 [134]	0.80 ± 0.25 [55]
Docosahexaenoic (22:6n-3)	2.13 ± 0.41 [135]	2.08 ± 0.33 [55]

* $\bar{x} \pm SD$; *n* in brackets.

< 0.0001). The mean concentration, as a percentage of the total fatty acids in adipose tissue and platelets are presented in Table 3.

Risk factors, medication, and fatty acids

The correlations between several CAD risk factors and the two CAD scores are shown in Table 4. Age showed the strongest association with the two CAD scores. Cigarette smoking was also positively associated with CAD. Associations between the established serum lipid risk factors and the CAD scores were

TABLE 4
Spearman's rank correlation coefficients describing the associations between coronary artery disease (CAD) risk factors and CAD scores

Risk factors	Extent score		Myocardial score	
	Men	Women	Men	Women
Age	0.29*	0.39*	0.42†	0.41*
Cigarette smoking	0.09	0.29‡	0.21‡	0.18
Body mass index	-0.16	0.09	-0.01	0.08
Total cholesterol	0.03	0.13	0.05	0.10
LDL cholesterol	0.10	0.15	0.11	0.13
HDL cholesterol	-0.05	-0.14	-0.05	-0.24‡
Triglycerides	-0.03	0.19	0.03	0.27‡

* $P < 0.001$.† $P < 0.0001$.‡ $P < 0.05$.

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LINOLEIC ACID AND CAD

231

generally weak and rarely significant, particularly for men. For men, none of the associations was significant. For women, HDL cholesterol was inversely associated with the myocardial score and triglycerides were positively associated with the myocardial score. For the total population, HDL cholesterol was significantly inversely associated with both the extent score ($r = -0.14$, $P < 0.05$) and the myocardial score ($r = -0.16$, $P < 0.05$). Both men and women classed as hypertensive had a significantly higher extent score ($P < 0.05$) and myocardial score ($P < 0.05$) than those without hypertension as a risk factor for CAD.

The correlations between several of the CAD risk factors and fatty acids in adipose tissue and platelets are presented in Table 5. The other risk factor that was related to several of the fatty acid measurements was hypertensive status. Men with hypertension had significantly higher adipose tissue linoleic acid ($P < 0.05$) concentrations than men without hypertension. Women with hypertension had significantly lower platelet linoleic acid than women without hypertension ($P < 0.05$). Dietary change by men was associated with a significantly higher concentration of adipose tissue linoleic acid ($P < 0.05$). Diabetic status was not associated with any of the fatty acids.

Treatment of the patients with aspirin, nitrates, beta-blockers, calcium antagonists, or angiotensin-converting-enzyme (ACE) inhibitors were also related to concentrations of several of the fatty acids. Aspirin was associated with significantly higher platelet eicosapentaenoic acid for men ($P < 0.05$) and higher

platelet linoleic acid ($P < 0.01$) and α -linoleic acid ($P < 0.05$) for women. Nitrates were associated with lower adipose tissue lauric acid ($P < 0.05$) for men and lower platelet stearic acid ($P < 0.05$) for women. Beta-blockers were associated with higher adipose tissue oleic acid for women ($P < 0.05$). Calcium antagonists were associated with higher concentrations of adipose tissue eicosapentaenoic acid ($P < 0.05$) and platelet docosapentaenoic acid ($P < 0.05$) for women. ACE inhibitors were associated with higher adipose tissue docosapentaenoic acid for men ($P < 0.01$).

The relationships between particular fatty acids in adipose tissue with the same fatty acid measured in platelets are presented in Table 6. In general, the associations between the same fatty acids in the different tissues were not strong. The fatty acid with the highest concordance between adipose tissue and platelets was linoleic acid.

Adipose tissue fatty acids and CAD

The relationships between adipose tissue fatty acids and the two CAD scores are presented in Table 7.

Men. Myristic acid was inversely associated with the myocardial score. This association is not significant after age was adjusted for ($r = -0.16$, $P > 0.05$). Palmitoleic acid was inversely associated with the extent score. After age, triglycerides, and cigarette smoking were adjusted for, the association remained significant ($r = -0.24$, $P < 0.05$).

Linoleic acid was positively associated with both the extent score and the myocardial score. After age was adjusted for, the

TABLE 5

Spearman's rank correlation coefficients describing the associations between fatty acids in adipose tissue and platelets and risk factors for coronary artery disease

Fatty acid	Men						Women					
	Age	Smoking	BMI	LDL-C	HDL-C	TO	Age	Smoking	BMI	LDL-C	HDL-C	TO
Adipose tissue fatty acids												
Lauric (12:0)	-0.13	-0.04	-0.17	0.03	0.09	-0.04	-0.17	-0.03	-0.30	-0.16	0.15	-0.18
Myristic (14:0)	-0.13	-0.02	-0.17	0.02	-0.02	0.11	-0.21	0.00	-0.38*	-0.22	0.05	0.11
Palmitic (16:0)	-0.03	0.01	-0.04	0.02	-0.10	0.31†	-0.11	0.18	0.04	-0.12	0.07	0.25
Stearic (18:0)	0.07	-0.21*	-0.23*	-0.20	0.05	-0.13	0.11	0.09	-0.50†	-0.14	0.27	-0.17
Palmitoleic (16:1n-7)	-0.21*	0.24*	0.06	0.00	-0.01	0.19*	-0.14	-0.09	0.15	0.08	-0.05	0.08
Oleic (18:1n-9)	-0.05	0.06	0.07	0.05	-0.01	0.01	-0.10	0.03	0.06	-0.05	-0.25	0.02
Linoleic (18:2n-6)	0.18	-0.09	0.02	0.07	0.04	-0.25†	0.29	-0.01	0.12	0.18	0.02	-0.09
Homo- γ -linolenic (20:3n-6)	0.13	0.09	0.32†	0.07	-0.07	-0.02	0.32*	0.06	0.45*	0.03	0.03	0.28
Arachidonic (20:4n-6)	-0.06	0.11	0.19	-0.12	0.02	0.08	0.07	-0.06	0.52†	0.07	0.06	0.17
Alpha-linolenic (18:3n-3)	-0.03	-0.16	0.05	0.05	0.07	-0.08	0.10	-0.04	-0.12	-0.01	0.27	-0.21
Eicosapentaenoic (20:5n-3)	-0.11	0.15	-0.02	-0.14	0.25*	-0.19	-0.21	-0.19	-0.34	-0.04	0.19	-0.54†
Docosapentaenoic (22:5n-3)	0.16	-0.02	0.04	-0.20*	-0.00	-0.00	0.42†	-0.19	0.23	-0.04	-0.00	0.28
Docosahexaenoic (22:6n-3)	-0.03	-0.02	0.12	-0.15	0.09	-0.07	0.34*	-0.05	0.17	-0.11	0.26	-0.01
Platelet fatty acids												
Palmitic (16:0)	-0.01	-0.10	-0.00	-0.14	0.07	0.11	0.44†	0.04	0.01	0.23	0.09	0.23
Stearic (18:0)	-0.07	-0.13	-0.18	0.08	0.13	-0.19*	0.01	-0.06	-0.44†	-0.01	-0.10	-0.02
Palmitoleic (16:1n-7)	0.22*	0.07	-0.03	-0.09	0.20*	-0.09	0.11	0.14	-0.20	-0.05	-0.05	0.06
Oleic (18:1n-9)	-0.11	0.08	0.03	-0.14	-0.15	0.23†	-0.04	-0.12	0.27	-0.06	0.10	0.13
Linoleic (18:2n-6)	0.11	-0.07	0.01	-0.09	-0.14	0.11	0.09	-0.11	-0.06	-0.21	0.09	-0.14
Homo- γ -linolenic (20:3n-6)	-0.06	0.07	0.12	0.04	-0.19*	0.18*	0.30*	0.04	0.02	0.14	0.05	0.20
Arachidonic (20:4n-6)	-0.20*	-0.04	0.14	0.16	0.03	-0.03	-0.25	-0.04	0.10	0.12	0.05	0.12
Docosapentaenoic (22:5n-3)	0.01	0.04	0.09	0.04	0.05	-0.06	-0.36†	-0.06	0.14	-0.24	-0.09	-0.12
Alpha-linolenic (18:3n-3)	0.07	-0.07	-0.08	0.06	-0.06	-0.01	-0.04	-0.12	0.20	-0.06	-0.10	0.07
Eicosapentaenoic (20:5n-3)	-0.14	-0.02	0.14	0.19*	-0.07	0.07	-0.15	0.19	0.01	-0.12	0.04	-0.10
Docosapentaenoic (22:5n-3)	-0.06	0.04	-0.04	0.17	0.02	-0.09	-0.17	0.00	-0.14	-0.15	0.26	-0.30*
Docosahexaenoic (22:6n-3)	-0.03	0.11	-0.05	0.08	0.04	-0.08	-0.18	0.00	0.13	0.09	-0.12	0.07

* $P < 0.05$.† $P < 0.01$.of the total
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0.10
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-0.24†
0.27†

TABLE 6

Spearman's rank correlation coefficients describing the associations between the adipose tissue fatty acids and the same fatty acids measured in platelets

Fatty acid	Men	Women
Palmitic (16:0)	0.07	-0.08
Stearic (18:0)	0.31*	0.24
Palmitoleic (16:1n-7)	0.22†	-0.01
Oleic (18:1n-9)	0.16	0.27
Linoleic (18:2n-6)	0.46‡	0.49§
Homo- γ -linolenic (20:3n-6)	0.18	0.45*
Arachidonic (20:4n-6)	-0.05	-0.22
Alpha-linolenic (18:3n-3)	0.06	0.23
Eicosapentaenoic (20:5n-3)	-0.33†	0.21
Docosapentaenoic (22:5n-3)	-0.11	0.15
Docosahexaenoic (22:6n-3)	-0.03	0.33†

* $P < 0.01$.† $P < 0.05$.‡ $P < 0.0001$.§ $P < 0.001$.

association between linoleic acid and the extent score was not significant ($r = 0.14$, $P > 0.05$). The association with the myocardial score remained ($r = 0.19$, $P < 0.05$). After all covariates that were related to adipose tissue linoleic acid were adjusted for, namely age, triglycerides, hypertensive status, and dietary change, linoleic acid was not significantly associated with the extent score ($r = 0.13$, $P > 0.05$), but the association with the myocardial score remained ($r = 0.20$, $P < 0.05$). Adipose tissue homo- γ -linolenic acid was also positively associated with both CAD scores. After age and BMI were adjusted for, homo- γ -linolenic acid was significantly associated with the extent score ($r = 0.27$, $P < 0.05$) but not with the myocardial score ($r = 0.09$, $P > 0.05$).

Women. Docosahexaenoic acid was positively associated with both CAD scores. However, after age and BMI were adjusted for, the associations between both the extent score ($r = 0.13$, $P > 0.05$) and the myocardial score ($r = 0.14$, $P > 0.05$) were not significant.

Platelet fatty acids and CAD

The relationships between the platelet fatty acids and the two CAD scores are presented in Table 8.

Men. A positive association between linoleic acid and both the CAD scores was found. These associations remained after age was adjusted for [$r = 0.25$, $P < 0.01$ (extent score), $r = 0.24$, $P < 0.01$ (myocardial score)]. Arachidonic acid was inversely associated with both CAD scores. After age was adjusted for, the associations between arachidonic acid and the CAD scores were not significant [$r = -0.14$, $P > 0.05$ (extent score), $r = -0.12$, $P > 0.05$ (myocardial score)]. Platelet arachidonic acid was also inversely associated with platelet linoleic acid concentration ($r = -0.34$, $P < 0.0001$). To determine whether the inverse association between linoleic acid and arachidonic acid for men could be explained by age, cigarette smoking, diabetic status, dietary change, plasma cholesterol concentration, or any other serum lipid measurement, the association for each of these factors was adjusted for. None of these variables were able to explain (even partially) this inverse association.

TABLE 7

Spearman's rank correlation coefficients describing the associations between adipose tissue fatty acids and coronary artery disease scores

Fatty acid	Extent score		Myocardial score	
	Men	Women	Men	Women
Lauric (12:0)	-0.09	0.01	-0.18	-0.01
Myristic (14:0)	-0.12	-0.15	-0.20*	-0.25
Palmitic (16:0)	-0.04	-0.12	-0.09	-0.16
Stearic (18:0)	0.00	0.08	-0.09	-0.01
Palmitoleic (16:1n-7)	-0.21*	-0.18	-0.10	-0.08
Oleic (18:1n-9)	-0.15	-0.03	-0.18	0.05
Linoleic (18:2n-6)	0.20*	0.24	0.25†	0.21
Homo- γ -linolenic (20:3n-6)	0.24*	0.04	0.21*	0.08
Arachidonic (20:4n-6)	-0.03	-0.11	0.12	-0.03
Alpha-linolenic (18:3n-3)	0.08	0.14	0.05	0.04
Eicosapentaenoic (20:5n-3)	0.22	-0.15	0.10	-0.24
Docosapentaenoic (22:5n-3)	0.10	0.11	-0.00	0.19
Docosahexaenoic (22:6n-3)	0.02	0.33*	-0.01	0.33*

* $P < 0.05$.† $P < 0.01$.

The long-chain ω -3 fatty acids were not significantly inversely associated with either of the CAD scores. However, after LDL cholesterol concentration and aspirin use were adjusted for, both of which were significantly related to platelet eicosapentaenoic acid as well as age, eicosapentaenoic acid was inversely associated with the myocardial score ($r = -0.18$, $P < 0.05$).

Women. Arachidonic acid was inversely associated with the myocardial score. After age was adjusted for, this association was not significant ($r = -0.22$, $P > 0.05$). The associations between the long-chain fatty acids and the CAD scores were not significant. After calcium antagonist use was adjusted for, which was related to the concentration of docosapentaenoic acid, docosapentaenoic acid was significantly inversely associated with both the extent score ($r = -0.28$, $P < 0.05$) and the myocardial score ($r = -0.37$, $P < 0.01$). After age was further adjusted for, the association with the extent score was not significant ($r = -0.24$, $P > 0.05$) but remained significant for the myocardial score ($r = -0.33$, $P < 0.05$).

TABLE 8

Spearman's rank correlation coefficients describing the associations between platelet fatty acids and coronary artery disease scores

Fatty acid	Extent score		Myocardial score	
	Men	Women	Men	Women
Palmitic (16:0)	-0.06	0.23	-0.04	0.19
Stearic (18:0)	-0.03	0.05	-0.16	-0.11
Palmitoleic (16:1n-7)	-0.02	0.06	0.04	0.11
Oleic (18:1n-9)	-0.03	0.01	-0.05	0.14
Linoleic (18:2n-6)	0.27*	0.00	0.26*	-0.08
Homo- γ -linolenic (20:3n-6)	0.09	0.02	-0.01	0.10
Arachidonic (20:4n-6)	-0.19†	-0.24	-0.19†	-0.28†
Docosapentaenoic (22:5n-3)	-0.08	-0.25	0.04	-0.20
Alpha-linolenic (18:3n-3)	0.17	0.01	0.12	-0.05
Eicosapentaenoic (20:5n-3)	-0.07	-0.01	-0.12	-0.10
Docosapentaenoic (22:5n-3)	-0.16	-0.19	-0.09	-0.24
Docosahexaenoic (22:6n-3)	-0.06	-0.12	-0.05	-0.05

* $P < 0.01$.† $P < 0.05$.

LINOLEIC ACID AND CAD

233

Relationship outcomes

A summary of the major relationship outcomes observed for adipose tissue and platelet fatty acids after confounding factors were adjusted for, is presented in Table 9.

Discussion

The myocardial score has been used routinely as a means for quantitatively presenting the information obtained in a coronary angiogram. This score takes into account both severity and location of coronary lesions. Because severity is measured, the score may relate to factors that complicate atherosclerotic lesions in addition to the atherosclerosis itself. It has one major disadvantage if the relationships with risk factors for CAD are being examined. The score is biased by lesion location. The extent score is an estimate of the aggregate degree of atherosclerosis in the coronary arteries. This score is not biased by lesion location, it is quantitative, and it has been shown to be correlated more strongly with CAD risk factors than with a vessel score or a stenosis score (17). The extent score and the myocardial score were significantly associated, but the degree of association suggests that different aspects of CAD are being assessed. However, in general, those patients with more of the myocardium threatened by CAD also had a higher percentage of their coronary arteries affected by atherosclerosis.

The results for men from both adipose tissue and platelets show a positive association between linoleic acid and CAD. Adipose tissue is a good indicator of long-term intake of linoleic acid (1, 2), whereas platelet fatty acids reflect shorter-term intake. A correlation of 0.46 (Table 6) between adipose tissue linoleic acid and platelet linoleic acid for men is consistent with the different time frame of the measurements. However, factors other than diet can also influence the concentration of linoleic acid in both adipose tissue and platelets. Those factors that affect platelet linoleic acid might not affect adipose tissue linoleic acid. Although the degree of association between linoleic acid in adipose tissue or platelets and the CAD scores for men was not high, it was stronger than many of the other recognized risk factors for CAD. Together the results from platelets, and adipose tissue in particular, are indicative of a positive association between linoleic acid intake and CAD.

Much of the evidence now available suggests an inverse relationship between linoleic acid and CHD (3-6). However, one study found that an increased intake of linoleic acid significantly increased the risk of new atherosclerotic lesions in human coronary arteries (8). Interestingly, the end point in this study was also angiographic. Studies finding an inverse relationship for adipose tissue linoleic acid have used either rates of mortality from CHD (3, 4), angina pectoris, or acute myocardial infarction (5, 6) as an end point.

If the positive associations observed between linoleic acid and CAD in this study are due to a positive association between linoleic acid intake and CAD, as suggested by our results, then one possible explanation for the inconsistent findings may be that high linoleic acid intake is a risk factor for coronary atherosclerosis, just as low linoleic acid intake would appear to be a risk factor for coronary events (3-6). The mean concentration of linoleic acid in the adipose tissue of the study population was 12.6% for men and 13.7% for women, which is considerably higher than the mean concentration of linoleic acid in the adipose tissue of European populations studied previously (3-6).

TABLE 9

Major relationship outcomes after confounding factors were adjusted for*

Men

- 1) Adipose tissue palmitoleic acid (16:1n-7) was inversely associated with the extent score ($r = -0.24$, $P < 0.05$).
- 2) Adipose tissue linoleic acid (18:2n-6) was positively associated with the myocardial score ($r = 0.20$, $P < 0.05$).
- 3) Platelet linoleic acid (18:2n-6) was positively associated with both the extent score ($r = 0.25$, $P < 0.01$) and the myocardial score ($r = 0.24$, $P < 0.01$).
- 4) Adipose tissue homo- γ -linolenic acid (20:3n-6) was positively associated with the extent score ($r = 0.27$, $P < 0.05$).
- 5) Platelet eicosapentaenoic acid (20:5n-3) was inversely associated with the myocardial score ($r = -0.18$, $P < 0.05$).

Women

- 1) Platelet docosapentaenoic acid (22:5n-3) was inversely associated with the myocardial score ($r = -0.33$, $P < 0.05$).

* Possible confounding factors were age and other variables associated with the particular fatty acids. r is Spearman's rank correlation coefficient.

Several mechanisms that might account for the positive associations between linoleic acid and the CAD scores can be proposed. Higher concentrations of linoleic acid may increase the risk of oxidative modification of lipoproteins. The oxidative modification of LDL in particular is understood to be a pathway to atherosclerosis (22). Another possibility is that linoleic acid was a marker for the intake of other factors in food that increase the risk of CAD. A further alternative is that the observed associations for linoleic acid do not relate to intake, but to factors that influence the metabolism of fatty acids in vivo. The results from this study, however, provide insufficient evidence to support any proposed mechanisms.

Adipose tissue palmitoleic acid was inversely associated with the extent score. This is indicative of an inverse association with intake. However, the relationship between intake of saturated or monounsaturated fatty acids and adipose tissue concentration is not as strong as that for linoleic acid. Associations between saturated or monounsaturated fatty acids measured in adipose tissue and CAD therefore do not provide strong evidence for a relationship with intake. Adipose tissue homo- γ -linolenic acid was positively associated with the extent score. This association may be related to intake or to the metabolism of linoleic acid in vivo. Several other significant associations between adipose tissue fatty acids and the CAD scores were observed. All of these associations could be explained by age and other covariates.

At a univariate level, not one of the long-chain ω -3 fatty acids was significantly inversely associated with the CAD scores. After confounding factors were adjusted for, eicosapentaenoic acid was inversely associated with the myocardial score for men and docosapentaenoic acid was inversely associated with the myocardial score for women. These results are supported by findings from Wood et al (6), who found that platelet docosapentaenoic acid was inversely associated with acute myocardial infarction, and eicosapentaenoic acid was inversely associated with angina pectoris (6). The results are also consistent with several other studies that suggest that fish intake may be beneficial for CHD risk (9-11). One of the most important mechanisms for the inverse relationship between the fish oils, or the ω -3 fatty acids, and CHD is likely to be the effects of ω -3 fatty acids on eicosanoid metabolism such that a more vasodilatory state with reduced

associations
disease scores
myocardial score

no	Women
8	-0.01
0*	-0.25
9	-0.16
9	-0.01
0	-0.08
8	0.03
5†	0.21
1*	0.03
2	-0.03
5	0.04
0	-0.24
0	0.19
1	0.33*

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Women
0.19
-0.11
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0.14
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0.10
-0.28†
-0.20
-0.03
-0.10
-0.24
-0.03

platelet aggregation results (23, 24). However, ω -3 fatty acids have been shown to reduce plasma triglyceride concentration (25-27) and blood pressure (28, 29), both of which may reduce the rate of progression of coronary atherosclerosis. The inverse associations between platelet eicosapentaenoic acid for men and docosapentaenoic acid for women, and the myocardial score provide further evidence for a relationship between the intake of this fatty acid and reduced risk of CAD. This is consistent with the proposition that an adequate intake of these fatty acids is beneficial to CAD. 2

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Letters to the Editor

Can linoleic acid contribute to coronary artery disease?

Dear Sir:

Hodgson et al (1) have chosen the wrong end of the natural history of coronary heart disease (CHD) to address such a question. They studied only people with angina pectoris and established CHD, one-third of whom had had a previous myocardial infarction and more than half of whom said they had changed their diet. Presumably they had adopted the conventional diet for CHD, which includes a relative increase of polyunsaturated fatty acids in Australia and in the United States. Bolton et al (2) in Scotland demonstrated a strikingly higher polyunsaturated fatty acid intake in men who knew they had CHD than in men with just diagnosed CHD.

Hodgson et al do not state whether they set out to investigate linoleic acid status or whether this was a fishing expedition in which 13 different fatty acids in adipose tissue and platelets were measured (but not *trans* fatty acids) and then related to two angiographic scores of severity and extent of coronary disease. Their abstract is misleading. They found the percent linoleic acid in adipose tissue was significantly associated with only one of the two coronary scores after adjustment (for dietary change, etc) in men and not in women. Put another way, adipose tissue linoleic acid was not significantly associated with either coronary disease score in women and not significantly associated with one of the two angiographic scores in men. In the same study total cholesterol, low-density-lipoprotein cholesterol and body mass index were not related to coronary disease score either and docosahexaenoic acid (22:6 ω -3) was positively related to the score in women.

Other investigators, as well as the Edinburgh group that Hodgson et al cite, have produced evidence that linoleic acid is negatively associated with CHD, ie, protective, both in prospective studies (3, 4) and in case-control studies of acute myocardial infarction (5, 6).

National intakes of linoleic acid have increased in countries, including Australia, that have experienced a large decline in CHD mortality (7-10). Linoleic acid is known to lower plasma low-density-lipoprotein cholesterol (11), reduce the tendency to thrombosis (12), and reduce the liability of the ischemic myocardium to dangerous arrhythmia (13).

The question was asked recently whether linoleic acid makes low-density-lipoprotein more liable to oxidation than oleic acid,

but what may occur in a simplified in vitro system has been not demonstrated in living people (14).

AS Truswell

Human Nutrition Unit
University of Sydney
New South Wales
2006, Australia

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LETTERS TO THE EDITOR

1419

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Reply to AS Truswell

Dear Sir:

The available evidence indicates that higher linoleic acid intakes reduce the risk of angina and myocardial infarction (1-4), and may be related to a reduction in coronary heart disease (CHD) mortality. A negative association between linoleic acid status and coronary artery disease (CAD) might therefore be expected, because CAD is associated with increased risk of death from CHD. However, the mechanisms involved in reducing the risk of angina, myocardial infarction, and death from CHD may relate to thrombosis and arrhythmia, rather than to coronary atherosclerosis. The two scores used to assess the degree of CAD in our study (5) presumably relate more to coronary atherosclerosis than to factors such as thrombosis, which may complicate atherosclerotic lesions and increase the risk of myocardial infarction and CHD mortality. Two studies have now assessed the relationship between linoleic acid status and an assessment of CAD (5, 6), and both have found a positive association. Dietary change toward increased linoleic acid intake in those with greater CAD is one possible explanation for the findings in our study (5). However, dietary change was not associated with significantly greater CAD scores. Retrospective dietary change is difficult to assess, and the data collected cannot address this question adequately. Studies that examine the relationship between linoleic acid and CAD in subjects without suspected CAD will reduce the likelihood of dietary change and overcome this problem. These studies, at the other end of the natural history of CAD, will probably be performed when less invasive methods for the assessment of CAD are available. The technique of coronary angiography is invasive and carries with it a small risk of mortality. In the mean time we have acquired what data we can within ethical boundaries. As far as relationships we have not found are concerned, we place less weight on them than does Truswell, given the possibility of type II statistical error.

Jonathan M Hodgson
Mark L Wahlqvist
John A Boxall
Nicholas D Balazs

Department of Medicine
Monash Medical Centre
Melbourne, Victoria
3168 Australia

Premenstrual syndrome does exist

Dear Sir:

In their letter to the editor (1), Fong and Kretch cite old references to support their skepticism about the existence of premenstrual syndrome or late luteal phase dysphoric disorder (LLPD). It should be noted that a more recent and large body of psychiatric and gynecologic literature has accumulated on this condition and that much consensus has developed on its diagnosis and therapy. In fact, LLPD has been entered in the third edition of the *Diagnostic and Statistical Manual of Mental Disorders* (2), and although the task is not complete, this should not imply that the condition does not exist.

Raja A Sayegh

Vincent Memorial Gynecology Service
Massachusetts General Hospital
Boston, MA 02114

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Linoleic acid and coronary artery disease

Dear Sir:

Hodgson et al (1) incorrectly state that their study is indicative of a positive relationship between linoleic acid intake and coronary artery disease. Such a conclusion could only be justified if they had observed lower linoleic acid concentrations in a healthy control population than in patients with coronary artery disease. This they could not do or have not done. What was shown was that the severity or extent of coronary artery disease was greater in those patients with higher concentrations of adipose or platelet linoleate.

What would be interesting to know is whether the question regarding changes in the habitual diet implied a decrease in saturated and an increase in polyunsaturated fatty acid intake. The omission of the correlation coefficient between dietary change and adipose linoleate in this paper is a pity. Also, a separate analysis of extent of coronary disease and adipose linoleate in those who are unlikely to have changed their diet would have been helpful. The question is then whether the lesions became more severe because of high dietary linoleate or whether those subjects with more severe coronary artery disease were more likely to adhere to dietary advice. Both scenarios seem equally possible. Hodgson et al's study cannot distinguish between these two.

Until we can make this distinction, it is somewhat premature to speculate about lipid peroxidation, particularly in the absence of data. The authors perhaps felt justified to do so because dietary intake of polyunsaturates, estimated by 24-h recall, was associated with an increased risk for coronary artery lesion development in the Cholesterol Lowering Atherosclerosis Study (CLAS) study (2). However, this study also showed that increased intake of monounsaturates was associated with an increased risk of new lesions.

The arguments about the relative merits of polyunsaturated and monounsaturated fatty acids will continue. But we should remember that most of the studies have used unrealistic diets with polyunsaturated-saturated fatty acid ratios of up to 6 or 7. What is needed is good scientific studies with diets that are advocated to prevent coronary heart disease. Until then we should be wary of making any recommendation to prevent lipid peroxidation that could be exploited for commercial rather than scientific gain.

Rudolph A Riemersma
Denise Perkins
Andrew J Brown
Jonathan Brown

Cardiovascular Research Unit
The University of Edinburgh
George Square
Edinburgh EH8 9XF UK

Letters to the Editor

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1. Hodgson JM, Wahlqvist ML, Boxall JA, Balazs ND. Can linoleic acid contribute to coronary artery disease? *Am J Clin Nutr* 1993;58:228-34.
2. Blankenhorn DH, Johnston RL, Mack WJ, El Zein HA, Vailas LJ. The influence of diet on the appearance of new lesions in human coronary arteries. *JAMA* 1990;263:1646-52.

Reply to RA Riemersma et al

Dear Sir:

We stated in our paper (1) that the results are "indicative of a positive relationship between linoleic acid and CAD." This statement should have provided information about the intended limits to the assertion. The population studied was highly selected and therefore not representative of the general population. The statement was not aimed at the general population. We believe the results are indicative of a positive relationship between linoleic acid intake and the degree of coronary artery disease (CAD) within the patient population studied.

We agree with Riemersma et al that our results provide no evidence for lipid peroxidation as a pathway to CAD. We have included this in our discussion together with other possible mechanisms for the observed associations for linoleic acid, which also have no supportive evidence from the data in this study. The mechanisms suggested may or may not explain the findings in this study.

It is particularly difficult to evaluate food intake to assess its relationships with CAD in a cohort exposed to pressures to change diet. Thus, dietary change, rather than actual diet, was included in the analysis as a discrete variable with two levels: 0 = no dietary change, and 1 = dietary change. A correlation coefficient between adipose tissue linoleic acid and dietary change could not be provided. A nonparametric method to compare mean linoleic acid concentration in the two dietary change groups was used (Wilcoxon rank-sum test). Dietary change was associated with significantly higher adipose tissue linoleic acid ($P < 0.05$). This information is provided in the results section of the paper.

Riemersma et al are also interested in a separate analysis of the relationship between adipose tissue linoleic acid and degree of CAD in those who did not make a conscious decision to change their diet; that is, the "no dietary change" group. The reason for this analysis would be to obtain some idea as to whether dietary change over time, in patients with more severe CAD, toward an increased intake of linoleic acid is responsible for the observed "age, triglyceride, hypertension, and dietary

950

LETTERS TO THE EDITOR

change-adjusted association" between adipose tissue linoleic acid and the myocardial score of CAD ($r = 0.20$, $P < 0.05$). Within the "no dietary change" group the "age, triglyceride, and hypertension-adjusted association" between adipose tissue linoleic acid and the myocardial score was 0.29 ($P = 0.06$). The degree of correlation is actually stronger within the "no dietary change" group, but because of the reduced numbers in this group the association is not significant. Past dietary change is very difficult to assess even by using a crude yes-no approach. The data collected in this study cannot resolve the question as to whether dietary change is responsible for the observed association between adipose tissue linoleic acid and the myocardial score.

Jonathan M Hodgson

Mark L Wahlqvist

John A Boxall

Nicholas Balazs

Department of Medicine
Monash Medical Centre
Monash University
Melbourne, Victoria 3168
Australia

Reference

1. Hodgson JM, Wahlqvist ML, Boxall JA, Balazs NDH. Can linoleic acid contribute to coronary artery disease? *Am J Clin Nutr* 1993;58:228-34.

variety with total mortality. Diets that lacked food variety, characterized by a low DDS, were associated with increased total mortality. Although the idea of measuring food variety is a relatively new concept, at least one previous study has examined the association between food variety and an assessment of disease (4). Wahlqvist et al (4), examined the relationship between food variety and macrovascular disease assessed by using a non-invasive ultrasound measurement. It was found that greater food variety was associated with less macrovascular disease in those with non-insulin-dependent diabetes mellitus and also in healthy control subjects. This was perhaps the first study to assess the relationship between food variety and a disease outcome.

Jonathan M Hodgson

Bridget Hsu-Hage

Mark L Wahlqvist

Monash University Department of Medicine
Monash Medical Centre
Melbourne, Victoria 3168
Australia

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Dietary diversity and health

Dear Sir:

A dietary goal that several countries have assumed is to achieve the inclusion of a variety of foods in the diet (1). This guideline has been included because nutrients essential for human life are not all found in one food, but amongst many. The concept that the human diet should include a variety of foods is an established tenet of nutrition science thinking. However, few studies have set out to test the hypothesis that increased food variety is associated with better health. Perhaps one of the main reasons for the lack of evidence for this hypothesis is the need for established methods for the assessment of food variety. An approach for the development and use of food variety scores has been described recently (2).

An assessment of food variety, or dietary diversity as it has been called by Kant et al (3), is necessary to address this hypothesis, which is stated as a dietary guideline. Although there are problems with methods for the assessment of food variety, a major test of the usefulness of food variety scores is their ability to predict health outcomes and whether relationships observed are consistent with biological explanations. Kant et al (3) have used a "dietary diversity score (DDS)" as an assessment of food variety, and have for the first time related an assessment of food

Reply to JM Hodgson et al

Dear Sir:

I appreciate Wahlqvist et al (1) and Hodgson et al (2) bringing their contributions in the field of food variety and health outcome to my attention. Our primary intent for using the dietary diversity score (DDS) reported in the paper (3) was to relate a measure of overall diet quality with total mortality. Because the DDS necessitates inclusion of all the major food groups, it served such a purpose, while enabling an evaluation of the hypothesis regarding variety among food groups and health outcome. Given the complexity of human diets and the numerous interrelationships among nutrients in their function and metabolism, I agree with Hodgson et al regarding the importance of the study of food-based measures of total diet quality and their association with health.

Ashima K Kant

Queens College
City University of New York
Flushing, NY 11367

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